EXPRESSION OF Bcl-2 AND Ki-67 IN ENDOMETRIAL LESIONS

Dissertation submitted in

Partial fulfilment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL, 2013.

DECLARATION

I hereby declare that the dissertation entitled **EXPRESSION OF Bcl-2 AND Ki-67 IN ENDOMETRIAL LESIONS** was done by me in the Department of Pathology at Coimbatore medical college, Coimbatore during the period from August 2011 to July 2012 under the guidance and supervision of **Dr. Arjunan M.D.,** Additional Professor, Department of Pathology, Coimbatore medical college, Coimbatore. This dissertation is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree in Pathology. I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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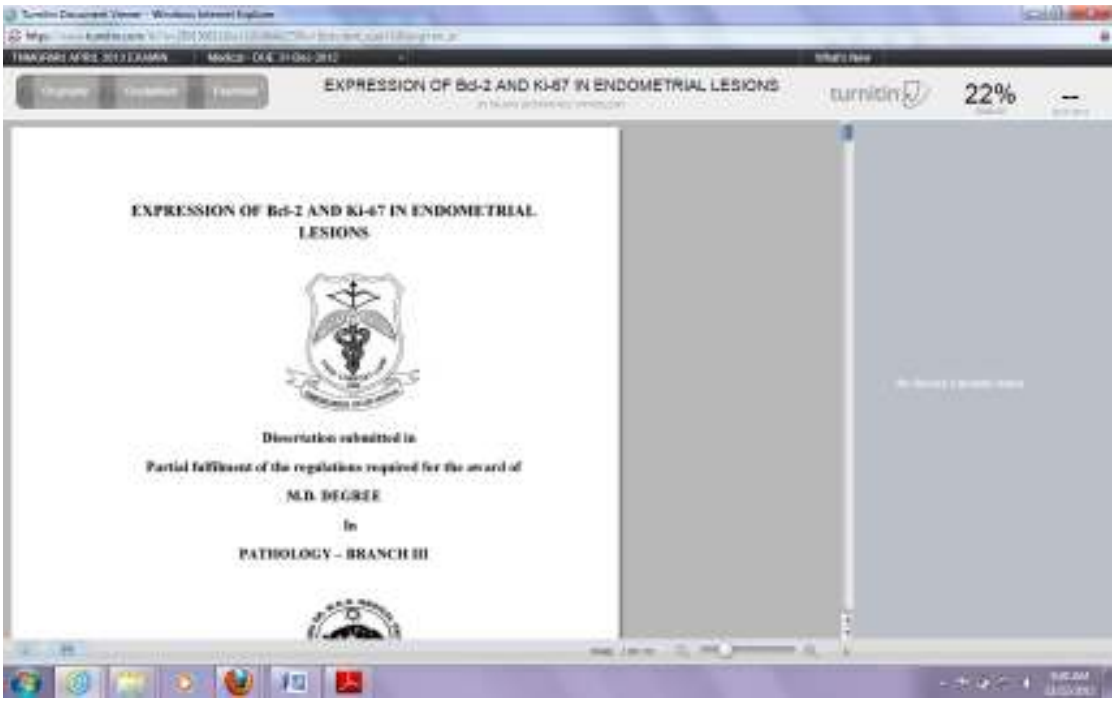

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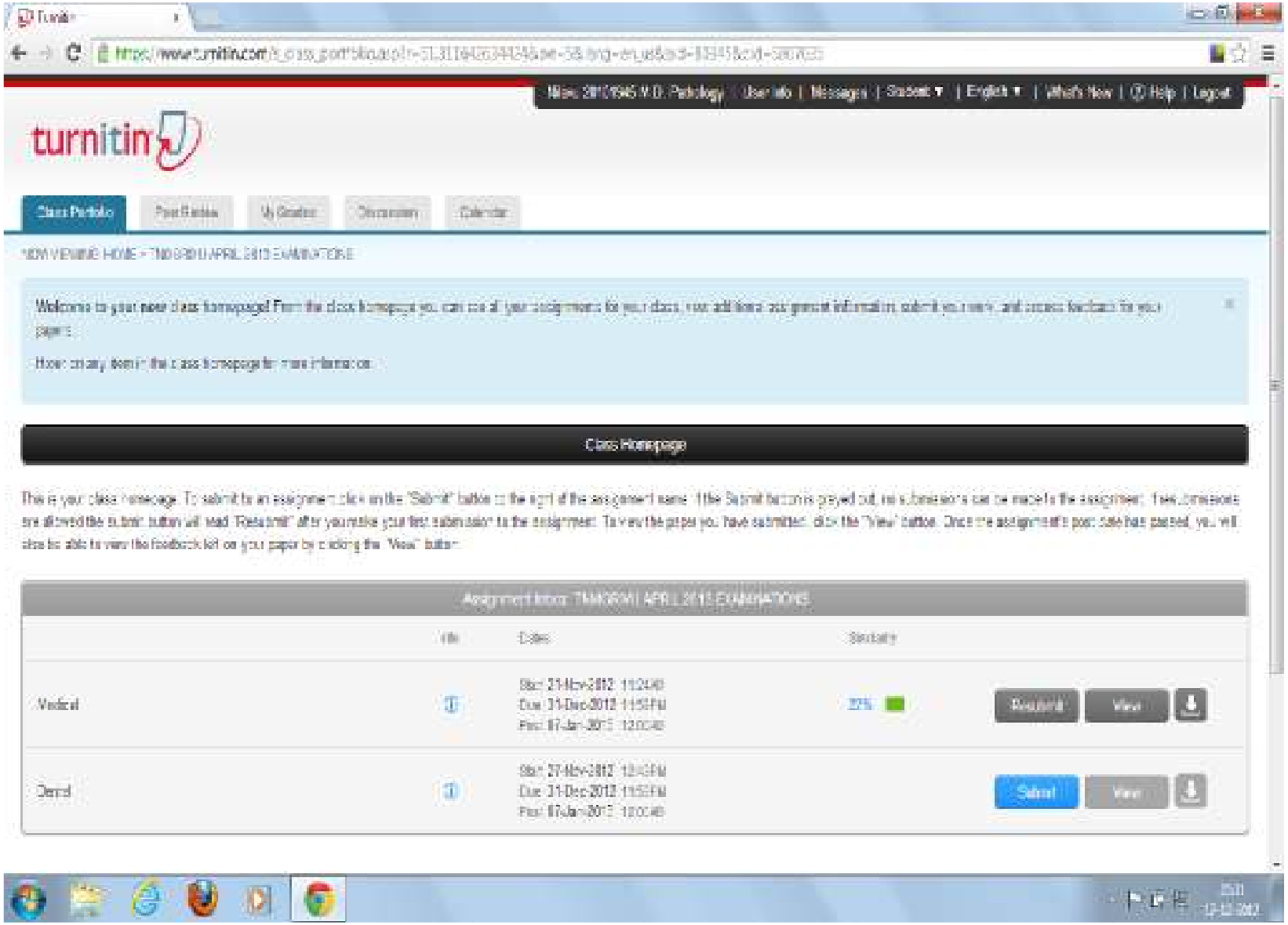
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ABBREVIATION

Bcl- B cell lymphoma.

EIN- Endometrial intraepithelial neoplasia.

TNF- Tumour necrosis factor

FADD- Fas associated death domain

DUB- Dysfunctional uterine bleeding.

HRT- Hormone replacement therapy

PAS- Periodic acid Schiff

IHC- Immunohistochemistry

MMMT- Malignant mesodermal (mullerian) mixed tumour.

INTRODUCTION

The human endometrium shows dynamic morphological changes during the menstrual cycle in reproductive women. These cyclical changes include cell proliferation with high mitotic activity, secretory changes and shedding of endometrial cells regulated by ovarian sex steroids. There should be some inhibitory mechanism to counterbalance such proliferation to maintain tissue homeostasis. Thus apoptosis which is programmed cell death is critical in regulation of the menstrual cycle.

There should be a balance between the apoptotic and mitotic activity for the regulation of normal menstrual cycle. The aim of this thesis is to study the apoptotic and mitotic activity in the endometrium of various lesions which includes cyclical endometrium , endometrial hyperplasias, premalignant conditions and malignancy. This is determined immunohistochemically by using markers such as Bcl-2 and Ki-67.

Bcl-2 is an anti-apoptotic gene involved in the regulation of apoptosis and Ki-67 is a recognized indicator of cell mitotic activity. Increased expression of both indicates tumorigenesis. Thus the pattern of expression in hyperplastic and premalignant states of endometrium helps us to study the progression of these conditions to frank malignancy.

AIM OF THE STUDY

The aim of this study is to study the apoptotic and mitotic activity in endometrium, the balance of which is essential for the regulation of normal endometrial development during the menstrual cycle. This is studied immunohistochemically by using markers such as Bcl-2 an anti-apoptotic gene involved in the regulation of apoptosis and Ki-67 a recognized indicator of cell mitotic activity in endometrial lesions such as cyclical endometrium, endometrial hyperplasia, premalignant conditions and malignancy.

OBJECTIVE

1. To determine the expression of Bcl-2 and Ki-67 in cyclical endometrium in the proliferative and secretory phases.
2. To study the expression of Bcl-2 and Ki-67 in hyperplastic states and premalignant conditions such as EIN.
3. To determine the pattern of expression of Bcl-2 and Ki-67 in endometrial carcinoma.

REVIEW OF LITERATURE

APOPTOSIS

The term apoptosis was coined by Kerr and Williams in 1972 who defined the process of cell death[1]. Apoptosis is a programmed cell death in which the cells die by activating enzymes that cause degradation of the cells nuclear DNA and cytoplasmic proteins[2]. The aim of the apoptosis is to eliminate the aged, unwanted and potentially harmful cells during the development and adulthood. It occurs in physiological conditions such as involution of hormone dependant tissues upon hormone withdrawal such as breakdown of endometrium during menstrual cycle, ovarian follicles undergoing atresia in menopause, the regression of lactating breast after weaning and pathological condition such as in neoplasm.

APOPTOSIS IN ENDOMETRIUM

The human endometrium consists of three phases of the cycle such as proliferative phase and secretory phase followed by menstrual phase. The follicular phase is characterized by rapid cell proliferation, which is predominently controlled by ovarian estrogens but also by other growth factors such as epidermal growth factor, fibroblastic growth factor, angiogenic growth factor etc. In the luteal phase, progesterone influences the secretory differentiation of the endometrium where the apoptosis

begins and levels increase with the forth coming menstruation.

PROCESS OF APOPTOSIS

The process of apoptosis may be divided into two phases such as initiation phase and execution phase. Apoptosis is initiated by two distinct pathways such as

1. Intrinsic or mitochondrial pathway.
2. Extrinsic or death receptor initiated pathway.

MITOCHONDRIAL PATHWAY

Mitochondrial pathway is the major mechanism of apoptosis. Mitochondria contains protein such as cytochrome-c that is essential for life, if released into the cytoplasm, they activate caspases which initiates apoptosis. This release of mitochondrial proteins is controlled by a finely orchestrated balance between pro and anti - apoptotic members of Bcl family.

THE BCL-2 PROTEIN

Caenorhabditis elegans, a nematode possess two genes ced-3 and ced-4, responsible for the programmed cell death, during the worm development and another gene ced-9 prevents their action. Bcl-2 (B cell lymphoma) gene was identified in human when it caused t(14:18) in most cases of follicular lymphoma, which promoted the survival of

cytokine dependant hematopoietic cell in the absence of cytokine [3]. Later Bcl-2 was found to have structure and function homologous to the ced-9 gene of the nematode and thus emerged as an anti-apoptotic gene.

There are more than 20 members of the Bcl family , and most of them function to regulate apoptosis. Certain proteins of this family are involved in anti-apoptotic mechanism the main ones being Bcl-2, Bcl-x, and Mcl-1. The pro apoptotic proteins are Bax, Bak and Bid. Thus anti apoptotic and pro apoptotic protein groups form homodimers and heterodimers between each other and resulting effect depends on the dominance of one of them. Bcl-2 is the best studied protein which acts as an antioxidant in the cell, protects cytochrome-c from being released from mitochondria and participates in the regulation of caspase proteins. Bcl-2 is situated in the outer membrane of the mitochondria and endoplasmic reticulum of the cytoplasm. It is also present in the nuclear envelope. Thus Bcl-2 controls the mitochondrial permeability and prevents leakage of mitochondrial proteins that have the ability to trigger cell death.

When cells are debilitated of survival signals or their DNA is damaged, or when misfolded proteins induce stress of endoplasmic reticulum, the sensors of damage or stress are activated. These sensors are also members of the Bcl family, and they include proteins called Bim,

Bid, and Bad that contain a single “Bcl -2 homology domain” and are called “BH-3 only proteins”. The sensors in turn activate two critical pro apoptotic effectors, Bax and Bak, which forms oligomers that insert into the mitochondrial membranes and create channels that allow proteins from the inner mitochondrial membrane to leak out in to the cytoplasm. BH-3 only proteins may also bind to and block the function of Bcl-2 and Bcl-x. At the same time Bcl-2 and Bcl-x synthesis may decline.

The net result of Bax-Bak activation coupled with loss of the protective functions of the anti-apoptotic Bcl family members is the release into the cytoplasm several mitochondrial proteins of which cytochrome–c, a protein involved in mitochondrial respiration binds to a protein known as Apaf-1(apoptosis activating factor-1). This complex binds caspase-9, the critical initiator of the mitochondrial pathway, and the enzyme cleaves the adjacent caspase molecules thus setting up auto amplification process.

EXTRINSIC PATHWAY

Extrinsic pathway is initiated by the involvement of the plasma membrane death receptor, the best one being type-1 TNF receptor. The cytoplasmic portion of this receptor contains a domain called death domain. Attached to this death domain is a related protein called Fas(CD95). The ligand for Fas is called the Fas ligand which is expressed

on the T cells that recognize self antigens. When FasL binds to Fas, more than three molecules are brought together and their cytoplasmic death domains form a binding site for the adapter protein called FADD(Fas-associated death domain), which in turn binds to caspase-8 in its inactive form. This complex combines with many caspase-8 molecules to generate active caspase, which initiates a series of caspase activation thus mediates the execution phase of apoptosis.

MORPHOLOGICAL CHANGES IN APOPTOSIS

Morphological changes taking place during apoptosis are cell shrinkage, condensation of chromatin, formation of cytoplasmic blebs followed by formation of apoptotic bodies. These apoptotic bodies are then phagocytosed by the macrophages which are then degraded by the lysosomal enzymes.

APOPTOTIC BODIES

On tissue sections apoptotic bodies appear as round or ovoid bodies with densely eosinophilic cytoplasm and fragments of chromatin. Because of the rapidity of the process of apoptosis such as cell shrinkage and phagocytosis, they are difficult to find in the histological sections. Further unlike necrosis it is not accompanied by inflammation. This also makes apoptosis difficult to be detected.

Thus the cyclical endometrial activity consisting of proliferation and menstrual shedding with increased apoptotic and mitotic activity has lead in the recent past years, immunohistochemical study of Bcl-2 in endometrium.

Ki-67 – THE NUCLEAR ANTIGEN[8]

The proliferative activity of a cell is determined by Ki-67 antigen. This monoclonal antibody was derived by immunizing mice with Hodgkins lymphoma cells. This is named after the city of origin, Kiel which is located in Germany. Ki-67 is predominantly present in the nuclear matrix and is expressed in all active phases of the cell cycle such as G1, S, G2 and M phase. It is absent in the G0 phase of the cell cycle, which are in the resting phase, and undetectable during the DNA repair process. It is particularly detected in the nucleus during the interphase stage of the cell division. Ki67 is involved in the regulation and maintenance of cell division. Expression of this nuclear antigen by a cell denotes that particular cell is the active phase of the cell cycle. MIB-1 is the antibody against different epitope of the similar proliferating antigen.

UTERINE CYCLE[4]

The mucosal lining of the uterus is composed of the glands and the stroma. The endometrium of the corpus is composed of two layer i.e stratum functionalis and stratum basalis. Stratum basalis is adjacent to

myometrium and consists of tubular glands and compact stroma. There is no secretory or mitotic activity in these glands. Stratum functionalis is futher divided in to superficial stratum compactum and the deep stratum spongiosum. Stratum spongiosum has maximal secretory activity and unresponsive stroma where as stratum compactum responds remarkably to hormonal stimulation with a prominent predecidual reaction and numerous granulocytes, where as the glands are stretched thin with less secretory activity.

Junction between the endometrium and the endocervical epithelium constitutes the isthmic endometrium. The isthmic endometrium consists of the glands with features between those of the endometrium proper and the endocervix. The glands are typically flattened and slit-like lined by an epithelium containing a mixture of undistinguished columnar cells admixed with ciliated cells. The stroma is more fibrous and displaying cells that are more spindled.

Normal menstrual cycle

Normal menstrual cycle is defined as bleeding from a secretory endometrium – associated with an ovulatory cycle- not exceeding a length of 5 days.[22] The menstrual cycle in the uterus is divided in to two main phases.

1. Proliferative phase
2. Secretory phase.

1. The normal proliferative phase[5]

This phase generally lasts two weeks but under physiological conditions, may fluctuate between ten and twenty days.

In the early part of this phase the endometrium is thin with sparse, small, straight glands and a loose stroma of spindle cells. At 8-10 days, the endometrial thickness increases, mainly as a result of stromal edema which peaks at day ten. In the preovulatory period as the estrogen levels starts decreasing, the stromal edema comes down which is overtaken by the continuous growth of the glands which becomes slightly tortuous.

Glandular epithelium is lined by tall columnar cells with basophilic cytoplasm and the nuclei are arranged in pseudostratified appearance staggered at varying heights, most in the basal half. The nuclei are ovoid with coarse chromatin. The endometrial stroma is densely cellular, consisting of cells that are small and oval with indistinct cytoplasm and hyperchromatic nuclei. Mitosis is frequent.

In the late proliferative phase the glands show increased tortuosity and variability in size. Mitosis becomes less numerous in the late proliferative phase. Subnuclear vacuoles and stromal edema starts to appear.

The secretory phase[4,6]

Ovulation as defined occurs on day 14 of the cycle. In first and second day of post ovulatory day, the endometrial glands appear slightly larger and less basophilic because of the interruption of the pseudostratified epithelium by vacuoles.

Early secretory phase

The appearance of the subnuclear vacuoles heralds the onset of early secretory phase which last from day fifteen to nineteen. The basal vacuoles push the nuclei to the middle of the cell giving a piano key like appearance. At seventeenth day there are uniform subnuclear vacuolation and no mitosis. Eighteenth day the vacuoles are moved toward the lumen accompanied by supranuclear discharge. Nineteenth day the nuclei again migrates back to the base. Mitotic activity is typically not present.

Mid secretory phase

The mid secretory phase last from day nineteen to twenty five. This is a phase of secretory exhaustion. At day twenty there is a peak intraluminal secretion. Day twenty one is characterized by an increase in stromal edema with prominent spiral arterioles. Day twenty two is characterised by a peak in stromal edema. By day twenty three the edema regresses and the stromal changes commence predecidualisation. By day

twenty four predecidua extends to bridge vascular elements. By day twenty five edema is progressively reabsorbed and glands in this phase are irregular and mitosis ceases to be apparent. Apoptotic bodies normally appear in the glandular epithelium in response to progesterone's down regulation of the proto-oncogene Bcl-2.[4]

Late secretory phase

Late secretory phase is from day twenty six to twenty eight. It shows extension of predecidual change from the surface lining downward deeply into the stratum compactum. By day twenty seven numerous granulated lymphocytes appear. In the central spongy part of the endometrium the glands have a characteristic saw tooth appearance.

Menstrual phase[7]

If pregnancy has not occurred, the late secretory phase leads inevitably to menstrual phase, starts 14 days after ovulation. This phase is recognized histologically by crumbling of the stroma and glandular collapse and hemorrhage in the superficial stroma. The stromal cells aggregate in to tightly clustered ball and separate from the glands, which have an unusually deep blue colour, and overlying epithelial cells appear degenerative. Also there is presence of plump epithelial glandular cells displaying secretory exhaustion. These tissue fragments are separated by red blood cells and neutrophils.

IMMUNOHISTOCHEMISTRY OF NORMAL ENDOMETRIUM[8]

- The endometrial glands and stroma are both positive for estrogen and progesterone receptors.
- Endometrial glands stain positive for Bcl-2, which is expressed high in the proliferative phase and decreased in the secretory phase.
- Endometrial glands are positive for cytokeratin 7 and negative for cytokeratin 20.
- Endometrial stroma is positive for CD10 and Bcl-2 and negative for CD34, in contrast to the cervical stroma.
- Stromal cells are positive for smooth mucle actin and negative for desmin.
- Granulated lymphocytes show positivity for CD56.

Many studies have been conducted using Bcl-2 and Ki-67 in normal cyclical endometrium. Reviews of these studies are as follows.

Mertens H J MM[9] et al states that Bcl-2 showed increased expression in the proliferative phase and diminished significantly in the secretory phase, especially in the glandular epithelial cells. Ki-67 also showed the same cyclical pattern with a later onset.

T. E. Vaskivuo[10] et al states that Bcl-2 showed increased expression in the proliferative phase and very low expression in the secretory phase.

Ki-67 was detected predominantly in the proliferative phase.

X J Tao[11] et al states that Bcl-2 immunoreactivity was maximal during the proliferative phase and decreased in the secretory phase.

A. Gompel[12] et al states that Bcl-2 expression was high in the glandular cells and peaked at the proliferative phase. Bcl-2 expression disappeared with the onset of secretory phase.

Pavel Havelka[13] et al states that increased expression of Bcl-2 protein was observed in artificial cycles in the surface epithelium and in the glands of the compact layer, as well as in the spongious layer.

R. Konno[14] et al states that the cyclic changes of survivin and Bcl-2 showed inverse relationship with Bcl-2 expression being maximum in the proliferative phase whereas survivin showed increased expression in the secretory phase.

Lydia J. Taylor[15] et al states that marked increase in the expression of Bcl-2 in the glandular epithelium and the stroma of the polyps in the proliferative phase in comparison with the proliferative endometrium and decreased in secretory phase polyps.

Hugo Maia[16] states that in endometrial polyps Ki-67, p53 and Bcl-2 expression was detected frequently during the proliferative phase than those in secretory phase of the cycle.

Rebacca K. Jones[17] et al states that rise in the stromal Bcl-2 expression is due to the up regulation of Bcl-2 expression by endometrial granular lymphocytes in the late secretory, premenstrual and early pregnancy endometrium. Ki-67 were high in the proliferative phase and early secretory phase and fell in the late secretory phase.

POST MENOPAUSAL ENDOMETRIUM[4]

After the cessation of estrogen and progesterone by the ovary, the endometrium is no longer stimulated and thus undergoes atrophy. The post menopausal endometrium is usually thin and inactive, although there is some variation in thickness.The glands are typically small and scant often unevenly distributed throughout the tissue. The glands are lined by cuboidal or flattened cells. The stroma becomes fibrous and the stromal cells lose their cytoplasm and appear cellular. Exposure to low levels of exogenous or endogenous estrogen, can result in a mildly expanded endometrium.

Another pattern of post menopausal endometrium is the cystic atrophy. This type of endometrium consists of cystically dilated glands, lined by flattened epithelium and the lumen contains partly non-specific secretion and partly transudate. This change may be so marked and may be visible grossly also called swiss cheese endometrium. The cystic atrophy arises as a result of benign hyperplasia present at the time of

menopause which regresses with withdrawal of hormonal support or simple obstruction of the gland ostia as a result of stromal fibrosis.

DYSFUNCTIONAL UTERINE BLEEDING

According to Ackerman, bleeding not associated with an organic cause in women of child bearing age belongs to the large and somewhat nebulous category known as dysfunctional uterine bleeding. The changes in the endometrium that confirm the patient has abnormal bleeding are the presence of fibrin clumps in the endometrial stroma, a finding usually not seen in the normal menstrual endometrium, the identification of fragmented pieces with dense stromal cellularity- a process known as stromal crumbling and the presence of increased number of apoptotic bodies at the base of the glands so called Bernirschke granules[22].

He classified DUB as ovulatory and anovulatory.

Ovulatory type is again classified as

1. Inadequate proliferative phase
2. Inadequate secretory phase
3. Irregular shedding of the endometrium

An anovulatory cycle results in prolonged unremitting estrogen stimulation which results in hyperplasia. All gradations of this phenomenon occur from disordered proliferative endometrium to an

atypical one that approaches the appearance of adenocarcinoma[66]. Davey D.A[67] defined DUB as an abnormal bleeding from the uterus in the absence of organic disease of the genital tract. Author classified DUB into primary and secondary.

Primary DUB- Primarily there is dysfunction in the pituitary, hypothalamus, ovary or uterus.

Secondary DUB - Factors such as IUCD insertion, hormonal contraception or secondary to systemic diseases such as endocrinopathies.

Primary DUB is classified as Ovulatory DUB and anovulatory DUB

Ovulatory DUB includes

1. Corpus luteal insufficiency
2. Persistent corpus luteum.

Anovulatory DUB includes

1. Insufficient follicular development
2. Persistent ovarian follicle

Classification of possible endocrine abnormalities associated endometrial histology and typical bleeding patterns in DUB

Type	Cycle	Endometrial histology	Type of bleeding
ovulatory	1. Short cycle- short proliferative phase. (ovarian hypersensitivity) 2.Long cycle- Long proliferative phase.(slow follicle development)	Normal endometrium Normal endometrium	Menorrhagia. Polymenorrhoea, Menorrhagia. Oligomenorrhoea,
Luteal insufficiency	1. Insufficiency – short luteal phase. 2.Persistent (Halban's disease)	Irregular ripening of endometrium or deficient secretory endometrium Irregular shedding of endometrium	Menorrhagia, Premenstrual spotting, Prolonged menstruation.
Anovulatory	1. Insufficient follicles short cycle(low estrogen level) 2.Persistent follicle or polycystic ovaries(high level of estrogen)	Inadequate proliferative or atrophic endometrium Proliferative or hyperplastic endometrium	Polymenorrhoea, menorrhagia. Metropathica haemorrhagica, Oligomenorrhoea,

DISORDERED PROLIFERATIVE ENDOMETRIUM[4,6,18,19]

Prolonged proliferation of endometrium as a result of anovulation gives rise to disordered proliferative endometrium. It is an intermediate between normal proliferative and benign hyperplastic endometrium. The patient usually presents with irregular uterine bleeding. It is characterised by cystically dilated endometrial gland distributed irregularly with stromal breakdown which is patchy, resulting from focal fibrin thrombus. Tubal metaplasia of endometrial glands is also common.

Rekha M D[20] et al states that there was a good correlation of the Bcl-2 expression and the apoptotic cell morphology in the different categories of the endometrium of dysfunctional uterine bleeding such as anovulatory, simple hyperplasia and secretory phase etc.

Zhou LL[21] states that the Bcl-2 protein expression changes periodically in normal cycle. The Bcl-2 expression intensity is enhanced in hyperplastic endometrium.

ENDOMETRIAL EPITHELIAL METAPLASIA[4,6]

Endometrial metaplasia is change in cellular differentiation of one type of epithelium, to another type that is not present in the normal endometrium. The most common forms of endometrial metaplasia are those in which the endometrial glands assume a morphology along the

mullerian differentiation such as serous, mucinous , or squamous. Metaplasia occurs in the benign endometrium, EIN and endometrial carcinomas.

SQUAMOUS METAPLASIA

Squamous metaplasia occurs in benign reactive process and carcinoma. The causes associated with benign changes are chronic endometritis, intrauterine devices, and trauma. There are two types of squamous metaplasia, such as

- Typical squamous metaplasia.
- Morular metaplasia.

Typical squamous metaplasia occurs as scattered surface foci. When whole of the endometrium is extensively replaced by squamous epithelium the condition is called ichthyosis uteri. This condition is associated with cervical obstruction and chronic inflammation.

Morular metaplasia occurs in the form cohesive, small, round and ‘ granuloma-like’ aggregates of squamous cells located in the glands or between the glands. The morules may enlarge into sheets with multiple glands arranged radially around the periphery. It can be associated with central necrosis. Sometimes morular metaplasia is associated with some degree of glandular crowding, where EIN has to be ruled out. The

transition of morular metaplasia to EIN is made by glandular crowding and altered cytological features within the glandular epithelium, as compared to the normal endometrium in other areas.

MUCINOUS METAPLASIA

Mucinous metaplasia is characterized by endometrial glands lined by cuboidal or columnar epithelium with prominent intracytoplasmic mucin , along with isolated pockets of extracellular mucin droplets. Goblets are seen occasionally. The presence of endometrial stroma around these mucinous glands differentiates from endocervical contamination in biopsy specimens. There are three types of mucinous metaplasia as follows

- Type A is characterised by glands lined by endocervical- like cuboidal epithelium, which is most commonly present in perimenopausal women.
- Type B metaplasia show architectural complexity and pseudopapillary pattern.
- Type C consisted of mucinous epithelium of glands arranged in cribriform pattern or exhibiting microglandular or villoglandular architecture. This pattern is associated with significant risk of adenocarcinoma.

CILIATED (TUBAL) METAPLASIA

The tubal metaplasia is characterised by the columnar epithelium lined by cilia, with clear round cells seen between the cells similar to the tubal epithelium and eosinophilic luminal borders. Normally ciliated epithelial cells are found in the proliferative phase and are associated with estrogen stimulation.

CLEAR CELL METAPLASIA

Clear cell metaplasia is characterized by epithelial cells with abundant clear cytoplasm. It is very rare and can occur in pregnancy. It should be distinguished from clear cell carcinoma by the following features.

FEATURES FAVOURING CLEAR CELL METAPLASIA OVER CLEAR CELL CARCINOMA.

- Normal architecture and distribution of the endometrial glands.
- Bland nuclear features.
- Metaplasia is usually present focally.
- There is no visible tumour made out grossly.
- Stromal invasion is not seen.
- Estrogen receptor is strongly positive.

HOBNAIL METAPLASIA

Hobnail metaplasia is rare and is caused by, degenerative changes associated with necrosis and exfoliation artefact. It is also closely associated with arias stella reaction. This metaplasia is characterised by glandular cells consisting of rounded nuclear protrusion beyond the cytoplasmic borders.

EOSINOPHILIC METAPLASIA

Eosinophilic or oxyphilic metaplasia is characterized by glands lined by cells with abundant granular eosinophilic cytoplasm. The abundant granular cytoplasm is due to the increased mitochondria.

Endometrial mesenchymal metaplasia includes cartilaginous and osseous metaplasia, smooth muscle metaplasia, glial metaplasia, and extramedullary hematopoiesis.

ENDOMETRIAL POLYPS

Endometrial polyps are biphasic growth of endometrial glands and stroma which protrude into the uterine cavity. It is one of the most common causes of abnormal uterine bleeding. Polyps arise as a result of monoclonal overgrowth of genetically altered stromal cells. Polyps are associated with hyperestrogenism. Their incidence is increased with HRT usage and tamoxifen therapy.

Microscopically polyp shows abnormally dilated glands with glandular crowding lined by atrophic epithelium or lined by poorly developed secretory or proliferative epithelium. The glands are set in a fibrous stroma. In some areas, the stroma shows marked hyalinisation. Characteristically the stroma contains thick walled blood vessels and sometimes thin ectatic vessels. Morphologically polyps are divided in to three types as follows.

- Proliferative or hyperplastic polyps consisting of proliferative, crowded glands.
- Atrophic polyps consisting of atrophic glands.
- Functional polyp consisting of glands resembling surrounding cyclical endometrium.

ENDOMETRIAL HYPERPLASIA

DEFINITION[8]

Endometrial hyperplasia is defined as increase in gland to stromal ratio, where there is estrogen induced proliferation of glands that are irregular in shape and size.

Endometrial hyperplasia is the morphological consequence of either,

1. Persistent follicle with high levels of estrogen for a long period ,

2. Repeated anovulatory cycles.
3. Excess of estrogen as a result of peripheral conversion of androgen as in polycystic ovarian syndrome.
4. Estrogen secreting ovarian neoplasm.

Patients with endometrial hyperplasia most frequently present with abnormal vaginal bleeding. Grossly hyperplastic endometrium is often characterised by abundant white to tan tissue that may have a diffuse or polypoid distribution and protrude in to the endometrial cavity. There are various classification of endometrial hyperplasia

Tavassoli and Krauss (1978)[68] classified as

Cystic hyperplasia

Adenomatous hyperplasia

Atypical hyperplasia.

Ronnett B.M and Robert J. Kurman(2004)[8] classifies endometrial hyperplasia as**1. Hyperplasia without cytologic atypia(non atypical hyperplasia)**

a) Simple

b)Complex

2. Hyperplasia with cytologic atypia(atypical hyperplasia)

a) Simple

b)Complex

Simple or complex types based on the degree of glandular crowding.

WHO[18] classification of endometrial hyperplasia depending upon both cytologic and architectural abnormalities (2003)

Hyperplasia without atypia

a) Simple hyperplasia without atypia

b) Complex hyperplasia without atypia

Atypical hyperplasias

a) Simple atypical hyperplasia

b) Complex atypical hyperplasia

HYPERPLASIAS[2,8,18,19]

Simple hyperplasia without atypia is characterized by slight increase in gland to stromal ratio. The glands exhibit marked variation in size and shape such as round and tubular glands to tortuous and cystically dilated glands (so called cystic or swiss cheese hyperplasia). The epithelial growth pattern and cytology are similar to that of proliferative

endometrium. The epithelial cells lining the glands are stratified and have oval nuclei with evenly dispersed chromatin and inconspicuous nucleoli. The stromal cellularity is increased as seen in proliferative phase. The blood vessels are dilated and are frequently thrombosed. Mitotic figures and apoptotic bodies are frequently noted. Approximately 1% of these lesions progress to adenocarcinoma.

Complex hyperplasia without atypia is characterized by increased gland crowding in comparison to simple hyperplasia. There is glandular enlargement and budding. The glands develop numerous branching channels and papillary infoldings. Cell stratification and polarity are generally maintained and the cells are cytologically normal. Mitotic activity is less than 5 mitosis per 10 high power field. Apoptotic bodies are frequently seen. About 3% of these lesions progress to adenocarcinoma.

Atypical hyperplasia

Cytological atypia is characterized by the presence of increased nuclear to cytoplasmic ratio and loss of polarity, with the nuclei being large and round. Nuclear pleomorphism, anisonucleosis and nuclear hyperchromasia is also seen. Nucleus is characterized by uneven distribution of the chromatin and prominent large nucleoli. Apoptotic bodies and mitosis are frequently seen.

Simple hyperplasia with atypia is a rare type of hyperplasia which has similar architectural features of simple hyperplasia, but is associated with cytologic atypia. About 8% of these lesions progress to carcinoma. **Complex hyperplasia with atypia** has architectural features similar to that of complex hyperplasia with epithelial cells showing cytological atypia. About 25% of these lesions progress to carcinoma.

Robert.J.Kurman[8] et al states that in general observations Bcl-2 expression is increased in simple hyperplasia, but decreased in atypical hyperplasias and invasive endometrial carcinomas[25,27,31,74-79].

Morsi, Hassan[23] et el states that Bcl-2 and Ki-67 show increased expression in proliferative endometrium, post menopausal endometrium and complex hyperplasia. Secretory endometrium showed decreased expression of both the markers. In endometrial carcinoma Ki-67 showed increased expression as the grade progressed, where as Bcl-2 reacted only weakly and only in grade 1 cancer.

Robert[24] et al states that, the Ki-67 mean index of 68 endometrial samples in descending order of frequency were as follows. Highest expression was observed in proliferative phase followed by complex hyperplasia, atypical complex hyperplasia, and simple hyperplasia. The least score was observed in atrophic endometrium.

Theodore H. Niemann[25] et al states that Bcl-2 protein expression was 100% in complex hyperplasia, 25% in complex atypical hyperplasia and 34% in cases of carcinoma. Complex atypical hyperplasia and carcinoma showed focal staining which was less in intensity than the normal proliferative endometrium.

Risberg .B[26] states that Bcl-2 showed highest expression in the proliferative phase, polyps and hyperplasias. Bcl-2 expression was decreased in secretory phases and carcinomas. Ki-67 expression was highest in carcinomas, proliferative phases than hyperplasia.

Olga B Ioffe[27] et al states that cytoplasmic Bcl-2 expression increased from proliferative endometrium to simple hyperplasia and decreased in complex hyperplasia and carcinomas. Ki-67 index was increased in proliferative endometrium and carcinoma and was decreased in simple and complex hyperplasia.

LBMora[28] states that Ki-67 index was higher in endometrial adenocarcinoma than in hyperplasia. Bcl-2 expression was lower in endometrial adenocarcinoma than hyperplasia and was higher in proliferative phase.

Altaner S[29] et al states that there was no statistically significant difference of Bcl-2 expression between the polyps, simple hyperplasia,

complex hyperplasia and endometrial adenocarcinoma. Positive Ki-67 was highest in endometrial adenocarcinoma followed by endometrial hyperplasia.

ENDOMETRIAL INTRAEPITHELIAL NEOPLASM[4,6.18]

Endometrial intraepithelial neoplasia is a clonal proliferation of architecturally and cytologically altered premalignant endometrial glands. Malignant transformation to endometrioid (type-I) endometrial adenocarcinoma is at a rate of 26 to 37%. The following factors are involved in the development of EIN such as

- Genetic mutations such as inactivation of PTEN a tumour suppressor gene is the most common genetic alteration found to occur in cancer preceded by EIN.
- PAX 2 inactivation and microsatellite instability.
- Activating mutations of the KRAS2 cellular oncogene.
- Other factors are unopposed estrogen, obesity, tamoxifen therapy etc.

EIN DIAGNOSTIC CRITERIA

1. Architecture- The gland area exceeds the stromal area. EIN lesions consist of aggregates of tubular glands or slightly branching glands, in which the surface area of the glands exceeds that of the stroma. The

crowded appearance of the glands are readily visualised under low magnificantion. These EIN glands have an epicentre with maximum glands concentrated in the periphery. The stroma in between the glands is hormone dependant which ranges from stroma of functionalis to more fibrous non cycling stroma of the basalis or polyps. The size of the glands and the distance between them determine the amount of stroma which is expressed as volume percentage stroma. In EIN the volume percentage stroma should be less than 55%. To evaluate this, ocular grids with 50-100 regularly placed points indicated by intersecting lines are used. Recently computerised histomorphometry is used to calculate the volume percentage stroma.

2. Cytology – The cytology of architectually crowded area is different from that of the background endometrial glands, where the crowded glands show round non-stratified nuclei or elongated pseudostratifed nuclei, with clumped or granular chromatin, with change in nucleoli. The cytoplasm may be of endometrioid differentiation or of non endometrioid differentiation such as tubal, mucinous, secretory or eosinophilic epithelium.

3.Size- An EIN lesion should be greater than 1mm in maximum dimension that usually encompasses >5-6 glands.

4. Exclusion of benign mimics - Features of EIN overlap with benign conditions which must be carefully discriminated from EIN itself.

- Endometrial polyps with irregularly placed glands and variable cytology are misinterpreted as EIN. But polyps usually exhibit altered stroma, thick vessels and randomly placed irregular glands.
- Benign endometrial hyperplasia should not be confused with EIN. Benign hyperplasia changes usually involve the entire endometrial compartment, and have an irregular random pattern of architectural and cytological alteration.

5. Exclusion of carcinoma- EIN are composed of clusters of individual glands with simple lining epithelium, whereas adenocarcinoma shows one or more patterns such as solid, cribriform, or complex interlacing maze like growth. These architectural changes of endometrial cancer reflect loss of basement contact between the epithelial cells.

Mahrosa M.M.Khedr[30] et al states that normal proliferative endometrium expressed high levels of both Ki-67 and Bcl-2. Ki-67 positivity sequentially increased from endometrial hyperplasia through EIN to endometrial carcinoma. In contrast Bcl-2 positivity was decreased significantly in cases of endometrial carcinoma mainly the high grade.

ENDOMETRIAL ADENOCARCINOMA[4,6,18,22]

Carcinoma of the endometrium is increasing in frequency compared with the carcinoma of the cervix. Endometrial cancers usually arises in postmenopausal women and 80% presents with postmenopausal bleeding. The peak incidence is in the 55 to 65 year old age group. Endometrial adenocarcinoma is broadly classified into two types, endometrioid (type I) estrogen dependant tumours and non-endometrioid (type II) adenocarcinoma less related to estrogen stimulation. The two main types of endometrial carcinoma exhibit different molecular alterations, consistent with the dualistic model of tumorigenesis[38] which is explained below.

TYPES OF ENDOMETRIAL ADENOCARCINOMA

Endometrioid type I estrogen dependant endometrial adenocarcinoma

Endometrioid tumours account for the majority of sporadic endometrial carcinomas (70-80%). In relation to the estrogen dependency these occur in younger, pre menopausal women. These tumours have low propensity for lymphatic spread and myometrial invasion than the non endometrioid adenocarcinoma. They are positive for estrogen and

progesterone receptors and generally carry a good prognosis. These tumours exhibit molecular alterations such as microsatellite instability, inactivated PTEN tumour suppressor gene, K- ras mutations, and abnormalities in b-catenin gene associated with b-catenin nuclear accumulation. [39]

Non- endometrioid type II estrogen independant endometrial carcinoma.

This type of tumour occurs in post menopausal women and account for 10-20% of endometrial carcinoma. They are not associated with the clinical evidence of estrogen stimulation and typically arise from atrophic endometrium, frequently in the setting of poorly differentiated phenotype of carcinosarcoma or undifferentiated carcinoma. They have rapid courses, a high degree of nuclear pleomorphism, frequent aneuploid DNA content, deeper myometrial invasion, increased lymphatic spread, low sensitivity to progestin and a poor prognosis. In contrast to estrogen dependant tumors these show loss of heterozygosity at different loci, altered p53, and abnormalities in genes regulating mitotic check points[40]

RISK FACTORS

Estrogens

Both exogenous and endogenous estrogen plays an important role in

the development of endometrial hyperplasia and carcinoma. From numerous case control studies it is evident that the threat of carcinoma in women taking unopposed estrogens is elevated 3 to 6 fold[28,29], rising to 9.5 fold if unopposed estrogens has been used for 10 years or longer[41]. Endogenous estrogen as in later menopause and low parity are the factors related to over all life time estrogen exposure.

Tamoxifen

Tamoxifen is a drug used in the chemotherapy of breast cancer. Its administration is associated with a slightly increased (two or three times) risk of endometrial adenocarcinoma.[42]

Polycystic ovary syndrome

Polycystic ovarian syndrome is a constellation of endocrine disorder sharing features of anovulation or infrequent ovulation , androgen excess and polycystic ovaries[43].The patients are usually infertile , have elevated estrogen levels, and associated insulin resistance may cause type 2 diabetes[44]. Endometrial carcinoma occurs in less than 5% of those women with polycystic ovaries .[45]

Obesity

Numerous studies firmly link obesity as a highly significant risk factor in women developing endometrial cancer,[46,47]. Adipose tissue is the

major site of aromatisation of androgen to estrogen and thus associated with increased risk of cancer.

Diabetes

3- 17% of the women with endometrial carcinoma has diabetes while 17-64% have an abnormal glucose tolerance test.

Ovarian lesions

Ovarian lesions are associated with prolonged excessive and unopposed estrogen production that sometimes causes benign endometrial hyperplasia, EIN, and endometrioid adenocarcinoma that is almost always low grade and early stage. The most common lesions include granulosa cell tumor, thecoma, polycystic ovary disease, and hyperthecosis. Diffuse ovarian hyperthecosis is characterized by the presence of luteinized cells dispersed singly or in aggregates throughout the hyperplastic stroma.

Sex cord stromal tumor

The incidence of endometrial carcinoma in patients with granulosa cell tumor is about 9-13%. Granulosa cell tumors are associated with endometrial hyperplasia and these patients presents with post menopausal bleeding.

Reproductive factors

Nulliparity is strongly associated with endometrial carcinoma in the pre menopausal age group. Infertility caused by anovulation and progesterone insufficiency is also associated with increased risk of endometrial carcinoma. Many studies have proved that even early menarche and late menopause are also associated with endometrial carcinoma.

CLASSIFICATION OF ENDOMETRIAL ADENOCARCINOMA[1](8)

ENDOMETRIOD ADENOCARCINOMA

Villoglandular

Secretory

Ciliated cell

Endometriod adenocarcinoma with squamous differentiation

CLEAR CELL ADENOCARCINOMA

SEROUS ADENOCARCINOMA

SQUAMOUS CARCINOMA

MUCINOUS ADENOCARCINOMA

MIXED TYPE OF CARCINOMA

UNDIFFERENTIATED CARCINOMA

[1] Modified WHO and International Society of Gynecological pathologist Histologic classification of endometrial carcinoma

GROSS FEATURES

The uterus may be slightly or grossly enlarged but it may be of normal size or even small and atrophic particularly in postmenopausal women. Most arise in the corpus and present either as a single mass or, two or three separate masses, or diffuse thickening of the endometrium. Carcinomas are frequently situated in the posterior wall. The most common appearance is that of a raised, rough perhaps papillary area of the endometrium with a shaggy surface and ulceration, frequently occupying at least half of the surface area of the endometrium. Sometimes the tumour is polypoid with a narrow base.

MICROSCOPIC FEATURES

The glandular pattern and cellular features generally resembles that of the proliferative endometrium showing a complicated architecture which includes solid growth, maze like interconnected lumen, villoglandular appearance or cribriform growth. Multilayered epithelial cells are nearly always seen. Solid growth may vary widely in extent which is an important feature in tumour grading. The individual epithelial cells are large, exhibiting nuclear stratification, coarsely clumped chromatin and prominent nucleoli. Individual tumours are associated with focal differentiation to squamous, mucinous, or tubal epithelium. Some endometrioid adenocarcinoma secretes abundant extracellular mucin, but

lack intracellular mucin which differentiates it from mucinous adenocarcinoma. Mitotic figures are less in well differentiated tumours.

Endometrial adenocarcinoma spread centrifugally in to the stroma. Foamy histiocytes are commonly seen in stroma of carcinoma endometrium. But the association between the foam cells and tumour grade is not yet proved. Myoinvasion is common and diagnosed only when the malignant glands have transgressed the endometrial myometrial junction in to the underlying muscular uterine wall.

International Federation of Gynaecology and Obstetrics (FIGO) recommended by WHO .

ARCHITECTURE GRADING

Grade 1 - 5% or less of non- squamous solid growth.

Grade 2- 6-50% of non-squamous solid growth.

Grade3- more than 50% of non – squamous growth.

CYTOLOGICAL GRADING[8]

Grade1- nuclei are oval, mildly enlarged, and have evenly dispersed chromatin.

Grade2- features intermediate to grades 1 and 3.

Grade3- nuclei are markedly enlarged and pleomorphic, with irregular coarse chromatin , and prominent eosinophilic nucleoli.

Note: Cytological features used in formulating the final grade

1. High degree of nuclear atypia not correlating with the architectural grade raises the grade1 or grade2 tumours by one level.

2. In carcinomas such as serous adenocarcinoma, clear cell adenocarcinomas and areas of squamous differentiation nuclear grade takes primacy over the architecture.

Low grade endometrioid carcinoma

The low grade endometrioid carcinoma consists of less than 5% of solid growth. These tumours are characterized by malignant glands lined by columnar cells similar to that of the normal proliferative endometrium, where the nucleus are arranged uniformly maintaining the polarity. But the glands are more irregular and larger than benign endometrium. Many of the variants comes under this category which includes, villoglandular variant, endometrioid adenocarcinoma with squamous differentiation, secretory variant, those showing mucinous differentiation and tubal differentiation .

Intermediate grade endometrioid carcinoma

These tumours are categorised as endometrial adenocarcinoma which has solid growth that constitute 5-50% of the tumour. The cytological grade is characterized by high degree of nuclear pleomorphism than grade-1 tumours, which includes, three to four times variation in the nuclear size and even more coarsely clumped chromatin.

High grade endometrioid adenocarcinoma

High grade endometrioid adenocarcinoma is characterized by the presence of solid growth greater than 50%. Some degree of solid growth pattern with higher nuclear grade pushes the grade to grade-3. Non-endometrioid tumours such as serous carcinomas, neuroendocrine carcinoma and undifferentiated carcinomas come under grade-3.

VARIANTS OF ENDOMETRIAL CARCINOMA(8)

Endometrial adenocarcinoma with squamous differentiation

Squamous differentiation ranges from keratinised epithelium that exfoliates anucleate squames to morules, which are cells with indistinct cytoplasmic borders arranged in sheets. Depending on the degree of differentiation of squamous cells, the term adenoacanthoma was used for the tumors with well differentiated squamous component and adeno squamous carcinoma for poorly differentiated squamous component.

Endometrial carcinoma without squamous differentiation and adenoacanthoma carry better prognosis than adenosquamous carcinoma.

Criteria for identifying squamous differentiation in endometrioid adenocarcinoma

Squamous differentiation is suggested by the presence of any one of the following[50]

- Keratin or keratin pearls demonstrated without special stain
- Intercellular bridges
- At least three of the following:

- Sheet-like growth without gland formation
- Distinct cell margins
- Deeply eosinophilic or glassy cytoplasm
- A lower nuclear cytoplasmic ratio than the surrounding tumour.

Endometrial adenocarcinoma with secretory differentiation

Secretory adenocarcinoma is a rare variant of endometriod adenocarcinoma consisting of glands resembling those of early or mid secretory endometrium[51]. The most common changes are subnuclear and supranuclear vacuolation. Secretary adenocarcinoma are associated with good prognosis[50].

Endometrial adenocarcinoma with ciliary cell differentiation

Ciliated cell carcinoma is rare[50,52]. Ciliated cells are uncommon in adenocarcinoma but occasionally individual ciliated cells can be found. More rarely, extensive ciliated cell is present throughout. A minimum of 75% of the cells should be ciliated to call it as ciliated cell carcinoma. Endometrioid carcinomas with ciliated cell differentiation have a good prognosis.

Endometrial adenocarcinoma villoglandular variant

This variant consists of slender, long, delicate papillae in well-differentiated neoplasm that may be predominantly of typical endometrioid type or entirely papillary[53]. This tumour is similar to villoglandular adenocarcinoma arising in the endocervix, where it occurs in the younger women and often associated with good prognosis. Psammoma bodies are rarely encountered[54]. The cytological features are that of a grade-1 carcinoma. Because these tumours are well differentiated they usually have a favorable outcome[55].

MUCINOUS ADENOCARCINOMA

Mucinous adenocarcinoma comprises 1-9% of all endometrial adenocarcinomas[31,32]. Mucinous endometrial adenocarcinomas usually arise in conjunction with an endometrioid component and thus can be

considered within the endometrioid class of endometrial adenocarcinoma[4].

A higher frequency of mucinous adenocarcinoma is observed in patients receiving tamoxifen and synthetic progesterone[33,34]. Grossly it is similar to endometrioid adenocarcinoma, apart from the infrequent prominence of the secreted tenacious mucus. Microscopically mucinous adenocarcinoma of the endometrium resembles mucinous tumour found in the endocervix. While many endometrioid carcinoma contain focal mucin, the mucinous component in mucinous adenocarcinoma should involve atleast half the cells[35]. The involved cells are tall with basal nuclei and prominent intracytoplasmic mucin. Mucicarmine, periodic acid schiff (PAS) and alcian blue are all useful to amplify the staining.

Most tumors are well differentiated (grade-1) but grade-2 and grade-3 tumours are occasionally described[31]. Poorly differentiated tumours lose their ability to produce mucin. Lymph node metastases may be well differentiated. In some cases exophytic complex filiform papillary fragments of mucinous endometrial adenocarcinoma are encountered in biopsy specimens. An extremely bland cytology, rare mitosis and a tendency to break apart, make these tumors difficult to recognize , as they resemble endocervical epithelium. The abundance of mucinous epithelium, delicate supportive stroma, and alternating mucinous and

non- mucinous differentiation often coexisting with a microglandular component are typical[36,37].

The behaviour of mucinous adenocarcinoma is that of endometrioid carcinomas. As most are well differentiated the prognosis is generally favorable.

SEROUS ENDOMETRIAL INTRA EPITHELIAL CARCINOMA

Endometrial intra epithelial carcinoma is the precursor of serous adenocarcinoma of the endometrium[56,40]. It typically occurs in post menopausal women in a background of atrophic endometrium. There is abrupt transition of this atrophic endometrium where the endometrial surface glands, are replaced by highly pleomorphic cells with marked nuclear atypia and mitotic activity, without invading the stroma. The cells express increased Ki67 index and a strong p53 immunoreactivity and loss of estrogen and progesterone receptors[40].

SEROUS ADENOCARCINOMA

Serous adenocarcinoma is an aggressive form of endometrial cancer accounting for 1-10% of all endometrial cancer. The patients are generally about 4-10 years older than women with endometrioid carcinomas, rarely have received estrogen therapy and lack previous or concurrent EIN or hyperplasia[57.] Most women are parous(90%). Few are

obese (10%) or have diabetes[58]. Typically they have normal serum estrogen levels [59]. Gross features are similar to endometrioid carcinomas, although many appear as bulky and necrotic masses[40].

Histologically they typically have branching papillae, lined by highly pleomorphic cells. The cells lining the papillae exhibit nuclear stratification, which forms tufts that can detach freely. The glandular structures are irregularly shaped and often lined by polygonal rather than columnar cells[60]. The nuclei are large, exhibiting high degree of nuclear pleomorphism and atypia containing macronucleoli. Mitosis figures are increased in number. There is considerable increase in the apotosis and numerous apoptotic bodies are seen. Atypical mitotic figures and tumour giant cells are common.

Psammoma bodies are encountered in one- third of the cases[61]. Myometrial invasion[62] is very common and it elicits granulation tissue type of response in the invasion site. The molecular changes of p53 mutation is seen in 80-90% of the serous carcinoma which on IHC shows diffuse and intense nuclear staining involving almost all tumor cells[63,40]. Ki67 immunohistochemistry shows a high labeling index(about 40%) in serous carcinoma and estrogen and progesterone receptors are absent or only weakly expressed in most tumors[64]. PTEN gene is intact and expressed in normal levels in most serous carcinomas[65].

CLEAR CELL ADENOCARCINOMA

Clear cell adenocarcinoma is also a prototype of type II endometrial carcinoma. It comprises about 1-6% of all endometrial carcinomas and occur at an older age (mean age 65-69years) than endometrioid adenocarcinoma [50.]. The women generally are less often obese, less often have often have diabetes mellitus, and less frequently have taken hormone replacement therapy.

These tumours are characterised by a variety of patterns, including papillary, tubulocystic and solid. Papillary pattern is the most common. The papillae are often small and rounded and frequently show hyalinised fibrovascular cores. The tumour cells exhibit prominent clear cytoplasm due to abundant glycogen or may display oxyphilic cytoplasm. Typical hobnail cells which frequently line papillae and tubules are characterized by a nucleus that bulges in to the lumen. Cells lining the cyst are commonly flattened are cuboidal. Intracytoplasmic hyaline bodies are very frequent and characteristic of this tumour. Mucin may be seen in the lumens of the tubules and cyst. However eosinophilic hyaline mucin droplets are seen as intracytoplasmic vacuoles – targetoid cells are a characteristic feature. The nuclei are pleomorphic with prominent nucleoli and mitotic figures are frequent. Rarely psammoma bodies may be found. In contrast to serous adenocarcinomas these are p53 negative.

MIXED ADENOCARCINOMA

Mixed adenocarcinomas consists of mixture of type-I endometriod adenocarcinomas or their variants (including mucinous adenocarcinoma), and type-II non- endometrioid carcinomas. The minor component should constitute at least 10% of the neoplasm. The prognosis depends on the most aggressive component[60].

MALIGNANT MESODERMAL(MULLERIAN) MIXED TUMOUR(CARCINOSARCOMA)

Malignant mesodermal mixed tumours constitute less than 5% of malignant tumors of the uterine corpus. The risk factors associated with MMMT are body weight, exogenous estrogen, nulliparity, tamoxifen therapy, and pelvic irradiation for various carcinomas. Grossly the tumour appears necrotic, fleshy, hemorrhagic polypoidal mass filling the uterine cavity. Extensive myometrial involvement is seen even grossly. Microscopically it shows biphasic pattern of the growth and high grade nuclear features. Epithelial component may present as any special type such as mucinous, clear cell, endometrioid, squamous and undifferentiated carcinoma. The stromal component can be homologous such as fibrosarcoma, stromal sarcoma and leiomyosarcoma or it can be heterologous consisting of liposarcoma, rhabdomyosarcoma, osteosarcoma and chondrosarcoma. This tumor carries worst prognosis.

UNDIFFERENTIATED CARCINOMA

Carcinomas that lack any evidence of differentiation after extensive sampling are classified as undifferentiated carcinoma. They constitute less than 2% of endometrial tumors[35,]. They are composed of large pleomorphic cells with prominent nuclear atypia. This is classified separately in to large cell and small cell type even though there is no difference in survival. The prognosis is poor. Other types of endometrial carcinoma are squamous cell carcinoma, transitional cell carcinoma, small cell carcinoma etc

Staging of endometrial carcinoma- International federation of Gynecologists and Obstetricians, 1988

Stage I- Tumour confined to the uterus

Ia: Tumour confined to the endometrium.

Ib: Tumour invades < 50% of the myometrial wall

Ic: Tumour invades > 50% of the myometrial wall

Stage II- Tumour extends in to the uterine cervix

IIa- Tumour extends into the endocervical epithelial surface or glands.

IIb- Tumour infiltrates the cervical stroma.

Stage III- Tumour extends outside the uterus

IIIa- Tumour involves serosa and /or adnexa, and/or positive ascitis, or/peritoneal washings.

IIIb- Direct extension or metastasis to the vagina.

IIIc- Pelvic and / or para-aortic lymph node metastases.

Stage IV-

IVa- Tumour invades bladder mucosa and /or bowel mucosa.

IVb- Distant metastases(excluding metastases to vagina, pelvic serosa or adnexa)

MYOMETRIAL INVASION

Invasive adenocarcinoma infiltrates the myometrium in the form of jagged, irregular branching glands which is surrounded by inflammed granulation tissue. Invasive glands exhibit microcystic, elongated and fragmented pattern within the myometrium. Involvement of adenomyosis by carcinoma should be distinguished from myoinvasion because the former does not imply worse prognosis than carcinoma limited to the endometrium. It is distinguished by the following features.

- Neoplastic glands in the adenomyosis usually have a blunt advancing front where as infiltrating carcinomas has a jagged infiltrating edge.
- Foci of adenomyosis are surrounded by hyperplastic myometrium a feature not present in myoinvasion.

- CD10 positive endometrial stromal cells distinguishes carcinoma involving adenomyosis from myoinvasion.

PROGNOSTIC FACTORS

1. **Stage of the disease**

2. Histological type

3. Histological grade

4. Myometrial invasion

5. Cervical involvement

6. Adnexal involvement

7. Lymphovascular involvement.

8. Positive peritoneal cytology

Other prognostic factors are DNA ploidy, diploidy is associated with higher disease free survival . Immunohistochemical staining for ER, Bcl-2, c-erbB2, p53 and Ki-67 has been suggested as important parameters in assessing prognosis in endometrial carcinoma[18]. Finally, a number of molecular alterations have also been proposed as putative prognostic factors for endometrial carcinoma including assessment of microsatellite instability and alterations in PTEN and B- catenin.

Poorly differentiated carcinomas, clear cell and serous carcinomas shows increased apoptotic bodies and thus increased apoptotic index in contrast to well differentiated endometriod carcinoma. Hence there is decreased Bcl-2 expression in these tumors. This decreased Bcl-2 expression is associated with various other prognostic factors such as PR negative status, increasing depth of invasion, and increasing FIGO stage [,72]. Two other studies correlated the loss of Bcl-2 protein to recurrence of the tumour and lymph node metastases. In a study which included 115 samples of endometrial adenocarcinoma, Ki-67 index was correlated with the cell type, FIGO stage and histological subtype. Thus it can be taken as a independant prognostic factor.

Yamauchi[66] et al states that expression of Bcl-2 progressively decreased from low grade to high grade carcinoma and Ki67 index increased from low grade to high grade carcinoma.

Kosmas[67] et al states that Ki67 immunocytochemistry showed type-II endometrioid adenocarcinoma showed higher expression than type-I carcinoma. High grade tumours showed increased Ki67 expression than low grade tumours. Ki67 index was higher in proliferative phase endometrium than that of grade1 and type-I endometrioid adenocarcinoma. In secretory phase the expression was markedly diminished.

Helga B. Salvesan[68] et al states that apart from age, mean vessel density, FIGO stage, p53 and Ki67 protein expression are independant prognostic factors.

Bozdogen[69] et al states that Bcl-2 expression was maximum in hyperplasia compared to carcinoma. In normal endometrium Bcl-2 staining showed increased intensity in the proliferative phase, but decreased in the early and mid secretory phase and reappeared in the late secretory phase.

Dahmoun M[70] et al states that Bcl-2 showed no correlation to apoptotic index. The Ki67 index was higher and more heterogenous in grade 2-3 tumours than grade1 tumour.

Halperin R[71] et al states that the endometriod grade1 to grade 2 cases were characteried by increased immunoreactivity for Bcl-2, where as serous papillary endometrial adenocarcinoma and poorly differentiated adenocarcinoma showed immunonegativity for Bcl2.

Wenxin Zheng [72] et al states that Bcl-2 staining intensity was higher in the proliferative endometrium and hyperplasia, which diminished in endometrioid adenocarcinoma and serous papillary adenocarcinoma.

MATERIALS AND METHODS

This study is done on endometrial specimens received by the department of pathology, Coimbatore Medical college during the period August 2011-July 2012.

INCLUSION CRITERIA

All endometrial biopsy and hysterectomy specimens received by the department of pathology.

EXCLUSION CRITERIA

1. Specimen not received in formalin.

2. Inadequate specimen.

METHODOLOGY

Sections are cut at 4 microns in thickness and hematoxylin and eosin staining was done. For immunohistochemistry sections of 4 microns thickness are cut and taken on coated slides, which are coated with gelatin –chrome alum mixture and kept in incubation at 58 degrees overnight. These unstained slides are used for running immunohistochemistry by a two step indirect technique.

PROCEDURE OF IMMUNOHISTOCHEMSITRY

METHOD: Two step indirect technique.

PRINCIPLES OF THE PROCEDURE:

Antigens in tissues and cells are detected by a two-stage process: the binding of the primary antibody to specific epitopes and the subsequent detection of this binding by a colorimetric reaction. Tissue sections are taken on coated glass slides, which are coated with gelatin – chrome alum mixture. The sections are then incubated overnight at 37 degree Celsius for dewaxing .Antigen retrieval is done by the microwave heating of tissue sections in buffer solution. The buffer used is tris EDTA and citrate buffer. In my study both Bcl-2 and Ki67 antigens are retrived by heating in tris EDTA buffer which has a pH of 9. The tissue sections are then treated with Peroxide-Block for blocking the endogenous peroxidise activity. Power block is to prevent the protein- protein interaction and reduce excessive background staining.

REAGENTS USED

1) Peroxide Block: 3%hydrogen peroxide in water.
2) Power Block Reagent: A highly effective universal protein blocking reagent. Contains casein and propriety additives in PBS with 15Mm sodium azide.

3) Chromogen: DAB-3,3'-diaminobenzidine.
4) Liquid DAB Substrate: Comprises Tris buffer containing the peroxide and stabilizers.
5) SuperEnhancer Reagent.
6) Pol-HRP Reagent.
7) Counter stain: Mayer's Hematoxylin.
8) Buffer solutions:

TRIS BUFFER: (ph -7.6)

TRIS Buffer salt : 0.605 gm

Sodium chloride : 8 gm

Distilled water : 1000 ml

1N Hydrochloric acid : 3 ml

CITRATE BUFFER: (ph-6.0)

Trisodium citrate : 2.94 gm

Distilled water : 1000 ml

1 N Hydrochloric acid : 5 ml

TRIS EDTA: (ph-9.0)

TRIS Buffer salt : 6.05 gm

Disodium EDTA : 0.744 gm

Distilled water : 1000 ml

PROCEDURE:

1) Deparaffinise the sections in xylene for 30 minutes.

2) Wash in absolute alcohol for 5 minutes with 2 changes.

3) Wash the slides in tap water for 10 minutes.

4) Rinse in distilled water for 5 minutes.

5) Antigen retrieval is done by placing the slides with tris EDTA buffer solution in microwave : Medium-10 minutes: High-10 minutes.

6) Cool to room temperature and rinsed in distilled water.

7) Wash in TBS buffer for 5 minutes with 2 changes.

8) Treat with Peroxide Block for 10 minutes.

9) Wash in TBS buffer for 5 minutes with 2 changes.

10) Treat with Power Block for 10 minutes.

11) Drain the slides and cover with primary antibody (supplied from BIOGENEX) for 2 hours.

12) Wash in TBS buffer for 5 minutes with 2 changes.

13) Cover the slides with Super Enhancer for 30 minutes.

14) Wash in TBS buffer for 5 minutes with 2 changes.

15) Apply HRP polymer reagent and leave for 30 minutes.

16) Wash in TBS buffer for 5 minutes with 2 changes.

17) Treat with DAB Chromogen with Substrate buffer for 5 to 8 minutes. Diamino benzidene gives a brown colour to the antigen antibody reaction site. This should be used with caution, because it is carcinogenic.

18) Wash in TBS for 5 minutes with 2 changes.

19) Wash the slides in tap water for 5 minutes.

20) Counterstain with Mayer's Hematoxylin for 1 minute.

21) Wash in tap water for 5 minutes.

22) Air dry and mount with DPX.

Tumour cells are scored positive if there is golden brown cytoplasmic staining in the neoplastic cells.

INTERPRETATION: Bcl2 positivity will be indicated by cytoplasmic positivity in glandular and stromal cells. Placenta is used as a control for Bcl-2 cytoplasmic grading, in which the syncytiotrophoblast cells stain for grade 4 positivity[20]. Ki67 positivity is indicated by nuclear positivity in glandular cells. The mean percentage of positive glandular cells for both Bcl-2 and Ki-67in the functional layer of endometrium will be determined by counting 1000 cells in 10 randomly selected high power fields. There is no standard grading system for the Bcl-2 antigen. This grading is done based on the journals[14,15,17,20,73].

Positivity for both Bcl-2 and Ki67 will be scored as

grade1 = <25%

grade 2= 25-50%

grade 3= 50-75%

grade4= 75-100%

Immunostaining intensity will be scored as

grade1= mild

grade2= moderate

grade3= strong

grade4= very strong

Weighted score = positivity * intensity.

Bcl-2 stains uniformly all glandular epithelial cells so number of cells showing positivity is always kept as grade 4. Ki67 only cells of very strong intensity are counted as positive so intensity is always kept grade 4. So 4 is kept constant. Bcl-2 is graded, mainly based on the intensity and Ki67 is graded, mainly based on positivity and both are multiplied by 4. Thus maximum score is 16, and both Bcl-2 and Ki67 is given a score out of 16.

TABLES AND CHARTS

Table-1, Distribution of cases

S.No	Endometrial lesions	Sample size	Percentage
1.	Proliferative phase	12	24%
2.	Secretory phase	9	18%
3.	Disordered proliferative endometrium	6	12%
4.	Hyperplasia	10	20%
5.	EIN	3	6%
6.	Carcinoma	10	20%
	Total	50	100%

A total of 50 endometrial samples were studied for Bcl-2 and Ki67 expression which included 24% of proliferative endometrium, 18% of secretory endometrium, 12% of disordered proliferative phase, 20% of hyperplasias, 6% of EIN and 20% of carcinoma endometrium.

Chart-1

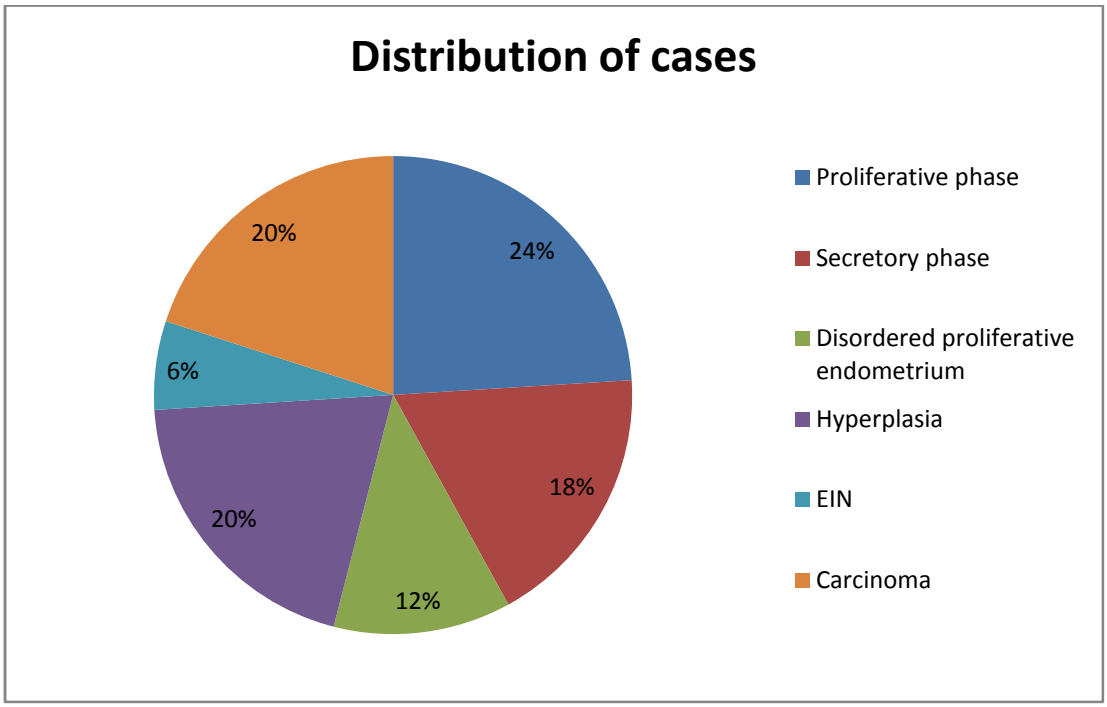


Table-2. Expression of Bcl-2 in various endometrial lesions.

S.NO	Endometrium lesions	Sample size	negative	Score 4	score 8	Score 12	Score 16	mean score
1.	Proliferative phase	12	-	3	4	4	1	9
2.	Secretory phase	9	6	2	1	-	-	1.77
3.	Disordered proliferative phase	6	1	2	2	1	-	6
4.	Hyperplasia	10	2	3	2	1	2	7.2
5.	EIN	3	-	-	2	1	-	9.3
6.	Carcinoma	10	5	1	-	2	2	6
	Total	50						

Bcl-2 showed maximum expression in proliferative phase and EIN, equal expression in carcinoma and disordered proliferative phase. Hyperplasia showed slightly higher expression than disordered proliferative phase. Secretory phase shows decreased expression compared to all lesions.

Chart-2, Expression of Bcl-2 in various endometrial lesions.

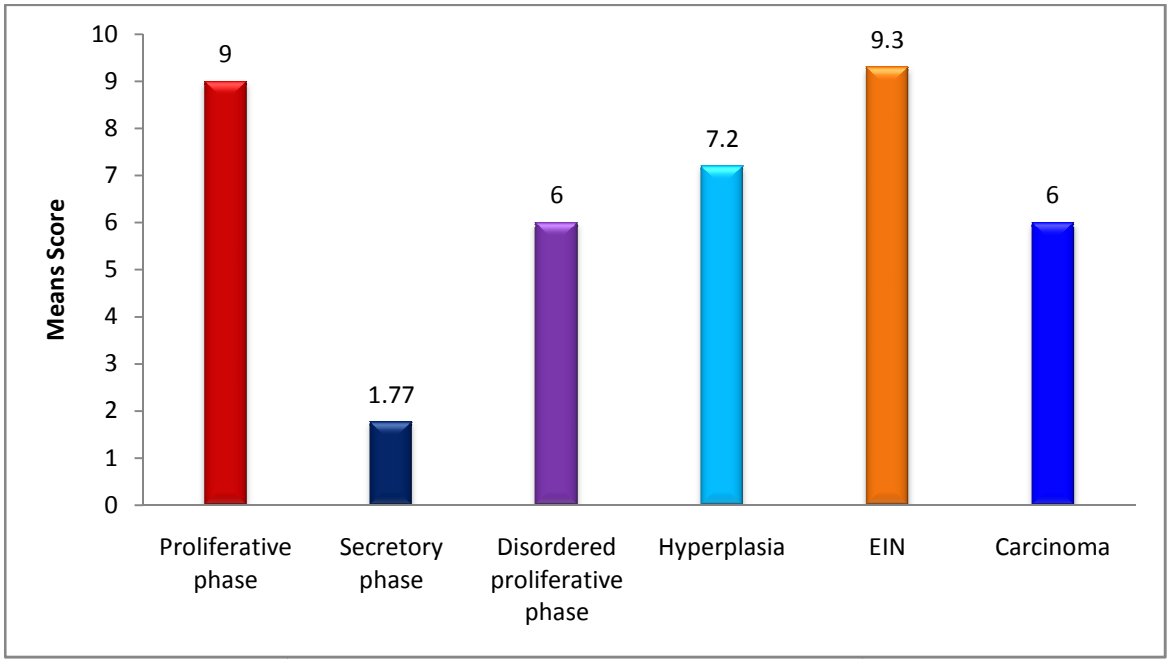
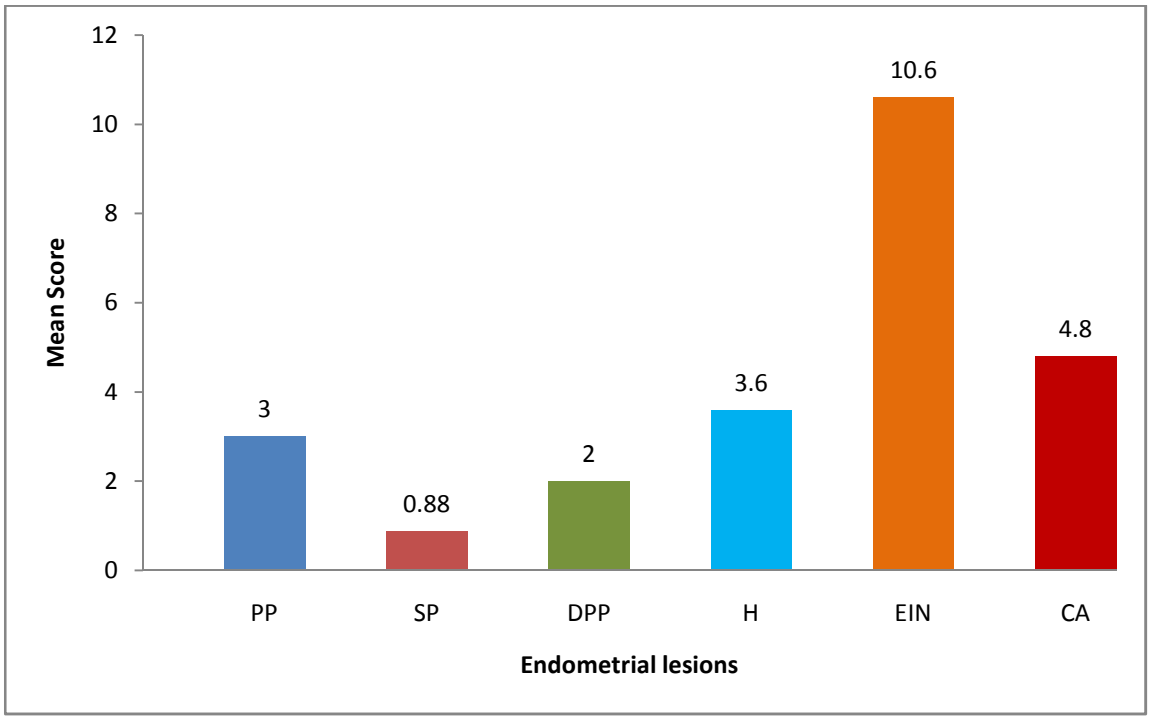


Table-3, Expression of Ki67 in various endometrial lesions

S. No	Endometrial lesions	Sample size	negative	Score 4	Score 8	Score 12	Score 16	Mean score
1.	Proliferative phase	12	4	7	1	-	-	3
2.	Secretory phase	9	7	2	-	-	-	0.88
3.	Disordered proliferative phase	6	3	3	-	-	-	2
4.	Hyperplasia	10	5	3	1	-	1	3.6
5.	EIN	3	1	-	-	-	2	10.6
6.	Carcinoma	10	5	1	2	1	1	4.8
	Total	50						

Ki-67 showed maximum expression in EIN followed by carcinoma. Hyperplasia and proliferative phase showed almost equal expression. The Ki67 expression was reduced in disordered proliferative endometrium and markedly decreased in the secretory phase.

Chart-3, Expression of Ki67 in various endometrial lesions



PP- Proliferative phase.

SP- Secretory phase

DPP- disordered proliferative phase.

H- Hyperplasia.

EIN- endometrial intraepithelial carcinoma.

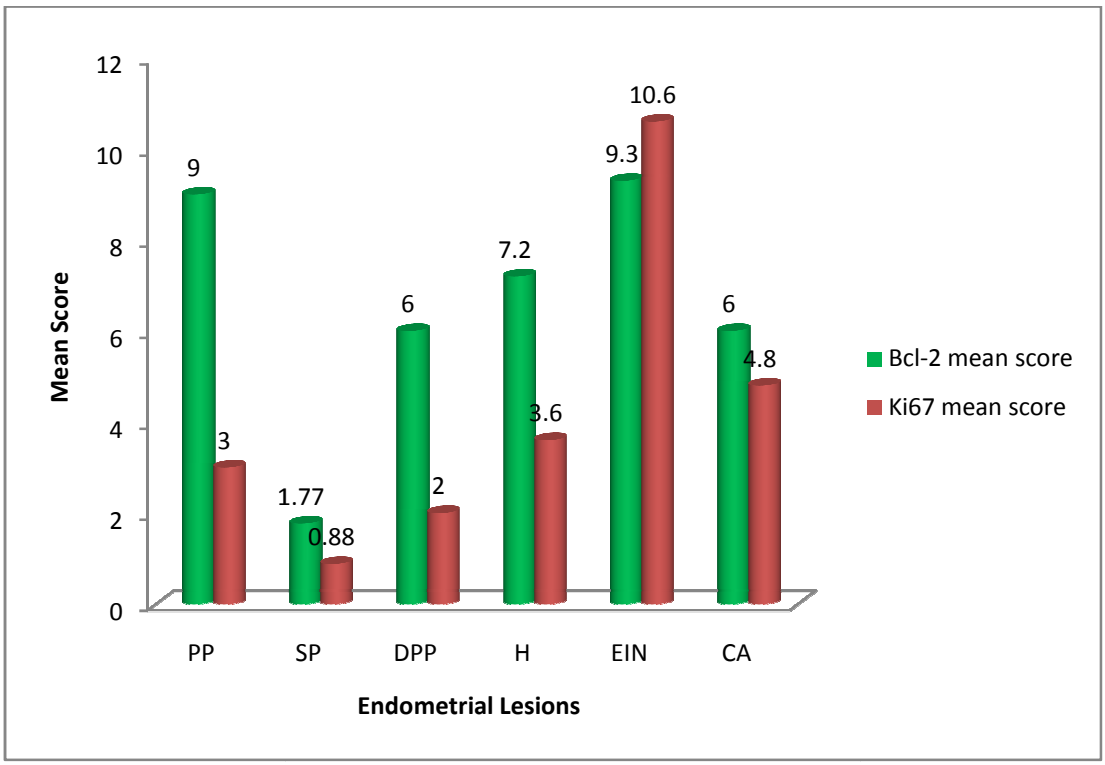
CA- carcinoma

Table-4, Comparison of Bcl -2 and Ki-67 in various endometrial lesions

S.NO	Endometrial lesions	Bcl-2 mean score	Ki67 mean score
1.	Proliferative phase	9	3
2.	Secretory phase	1.77	0.88
3.	Disordered proliferative phase.	6	2
4.	Hyperplasia	7.2	3.6
5.	EIN	9.3	10.6
6.	Carcinoma	6	4.8

Both Bcl-2 and Ki67 showed highest expression in EIN and lowest expression in secretory phase. Bcl-2 showed higher expression in proliferative phase, disordered proliferative endometrium and hyperplasia than Ki-67.

Chart-4, Comparison of Bcl - 2 and Ki-67 in various endometrial lesions



PP- Proliferative phase

SP- secretory phase

DPP- disordered proliferative phase

H- Hyperplasia.

EIN- Endometrial intraepithelial carcinoma.

CA- carcinoma

Table-5, Expression of Bcl-2 and Ki67 in cyclical endometrium

S.No	Endometrial phase	Sample size	Bcl-2 mean score	Ki67 mean score
1.	Proliferative phase	12	9	3
2.	Early Secretory phase	3	5.33	2.66
3.	Mid secretory phase	3	0	0
4.	Late secretory phase	3	0	0

Both Bcl-2 and Ki67 expression was high in the proliferative phase and showed decreased expression in the early secretory phase. In the mid and late secretory phase both markers showed immunonegativity.

Chart-5,Expression of Bcl-2 and Ki67 in cyclical endometrium

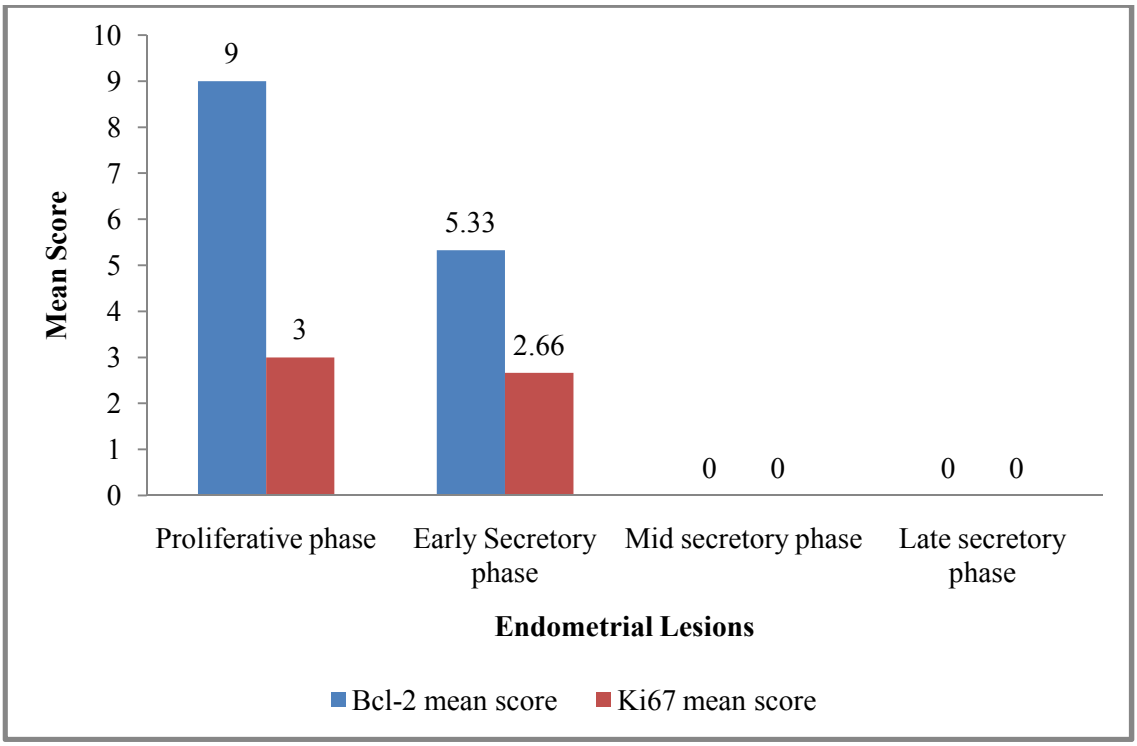
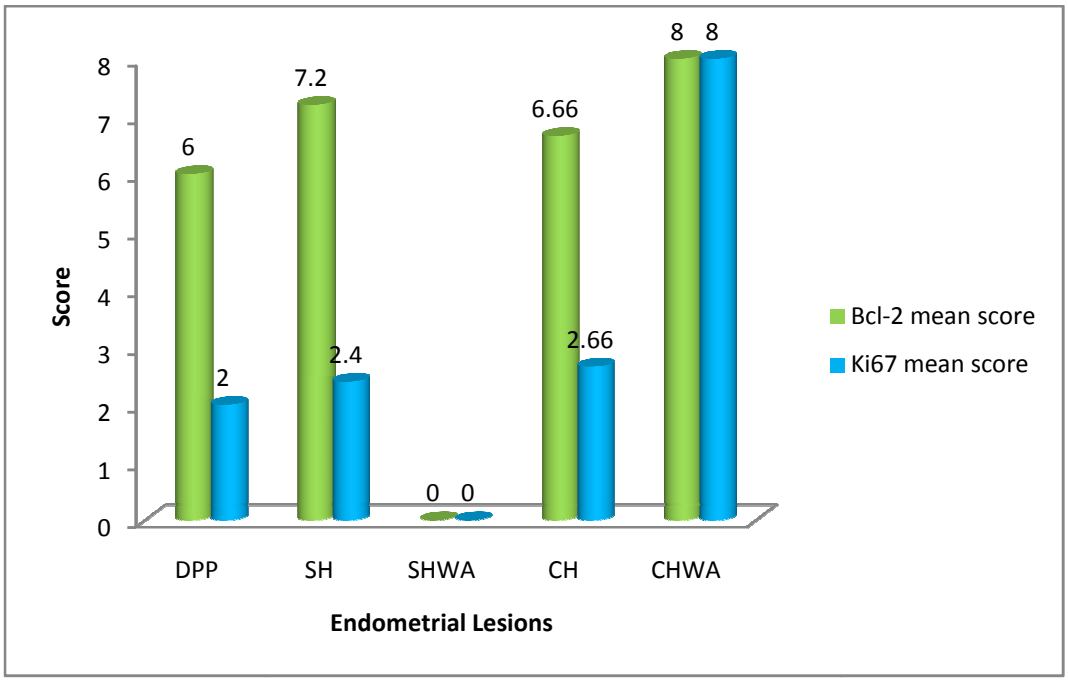


Table-6, Expression of Bcl-2 and Ki67 in disordered proliferative phase and hyperplasia

S. No	Type of endometrium	Sample size	Bcl-2 mean score	Ki67 mean score
1.	Disordered proliferative phase	6	6	2
2.	Simple hyperplasia without atypia	5	7.2	2.4
3.	Simple hyperplasia with atypia	-	-	-
4.	Complex hyperplasia without atypia	3	6.66	2.66
5.	Complex hyperplasia with atypia	2	8	8

Out of the 6 cases of disordered proliferative endometrium Bcl-2 showed positivity in 83.3% of the cases and Ki67 showed positivity in 50% of the cases. Both Bcl-2 and Ki67 showed highest score in complex hyperplasia with atypia and almost equal expression in simple and complex hyperplasia.

Chart- 6,Expression of Bcl-2 and Ki67 in disordered proliferative phase and hyperplasia



DPP- disordered proliferative phase

SH- Simple hyperplasia

SHWA- Simple hyperplasia with atypia

CH- Complex hyperplasia

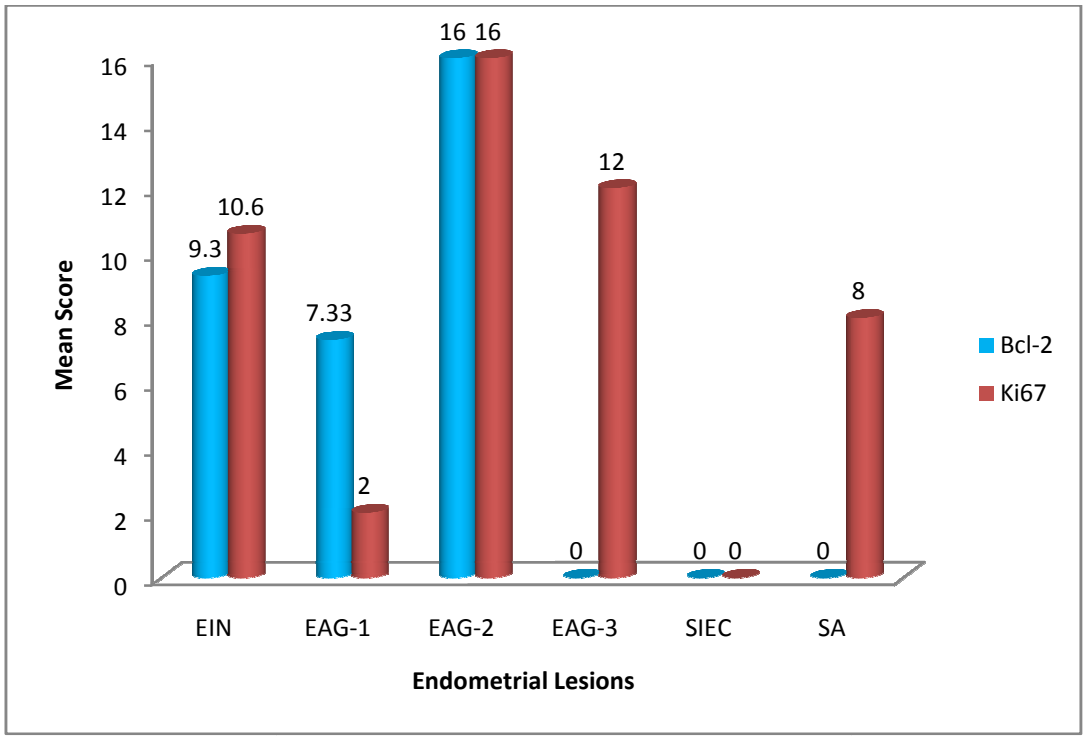
CHWA- Complex hyperplasia with atypia

Table-7, Expression of Bcl-2 and Ki67 in EIN and Carcinoma

S. NO	Endometrial lesions	Sample size	Bcl-2	Ki67
1.	EIN	3	9.3	10.6
2.	Endometrial adenocarcinoma grade -1	6	7.33	2
3.	Endometrial adenocarcinoma grade-2	1	16	16
4.	Endometrial adenocarcinoma grade-3	1	0	12
5.	Serous intraepithelial carcinoma	1	0	0
6.	Serous adenocarcinoma	1	0	8

3 cases of EIN was studied and both Bcl-2 and Ki-67 showed maximal expression in EIN compared to all endometrial lesions. Bcl-2 expression was maximum in grade1 and grade 2 tumours and was immunonegative for grade 3 carcinoma, serous EIC and serous adenocarcinoma. Ki67 expression showed maximum expression in grade 2 and grade 3 tumours and its expression decreased in grade 1 endometrioid adenocarcinoma

Chart-7, Expression of Bcl-2 and Ki67 in EIN and Carcinoma



EIN- Endometrial intraepithelial neoplasia.

EAG-1- Endometrial adenocarcinoma grade-1

EAG-2- Endometrial adenocarcinoma grade-2

EAG-3- Endometrial adenocarcinoma grade-3

SIEC- Serous intraepithelial carcinoma

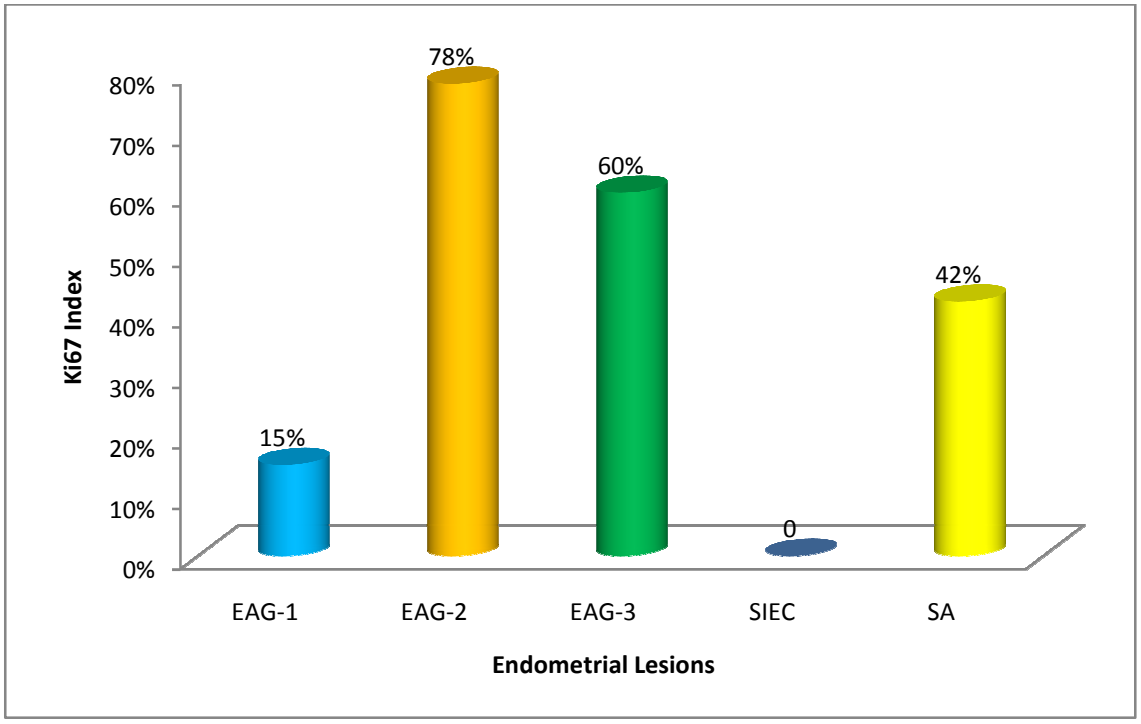
SA- Serous adenocarcinoma.

Table-8,Expression of Ki67 index in Carcinoma

S. NO	Endometrial lesions	Sample size	Ki67 mean index
1.	Endometrial adenocarcinoma grade -1	6	15%
2.	Endometrial adenocarcinoma grade-2	1	78%
3.	Endometrial adenocarcinoma grade-3	1	60%
4.	Serous intraepithelial carcinoma	1	0
5.	Serous adenocarcinoma	1	42%

Ki67 expression showed maximum expression in grade 2 and grade 3 tumours and its expression decreased in grade 1 endometrioid adenocarcinoma.

Chart-8,Expression of Ki67 index in Carcinoma



EAG-1- Endometrial adenocarcinoma grade-1

EAG-2- Endometrial adenocarcinoma grade-2

EAG-3- Endometrial adenocarcinoma grade-3

SIEC- Serous intraepithelial carcinoma

SA- Serous adenocarcinoma.

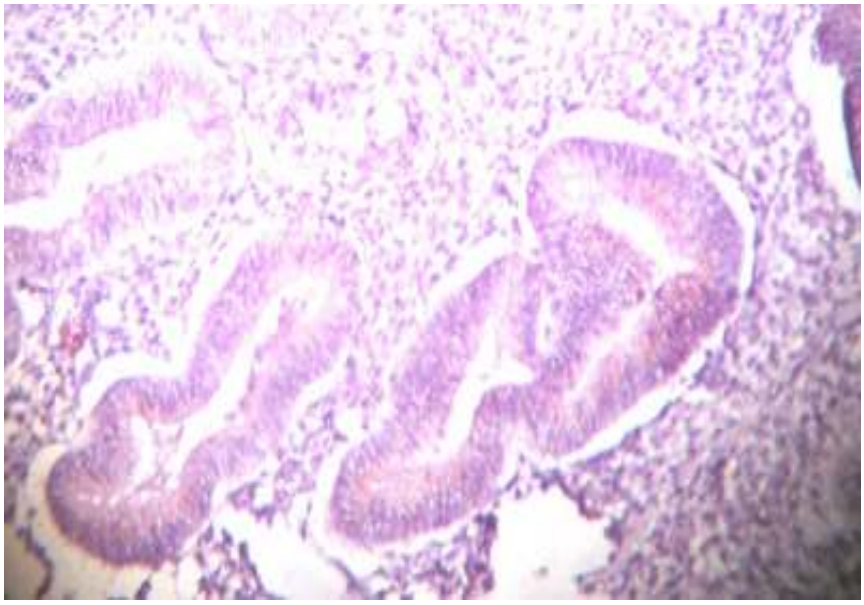


Fig-1, Proliferative phase showing cytoplasmic positivity of Bcl-2 grade-1, 10X

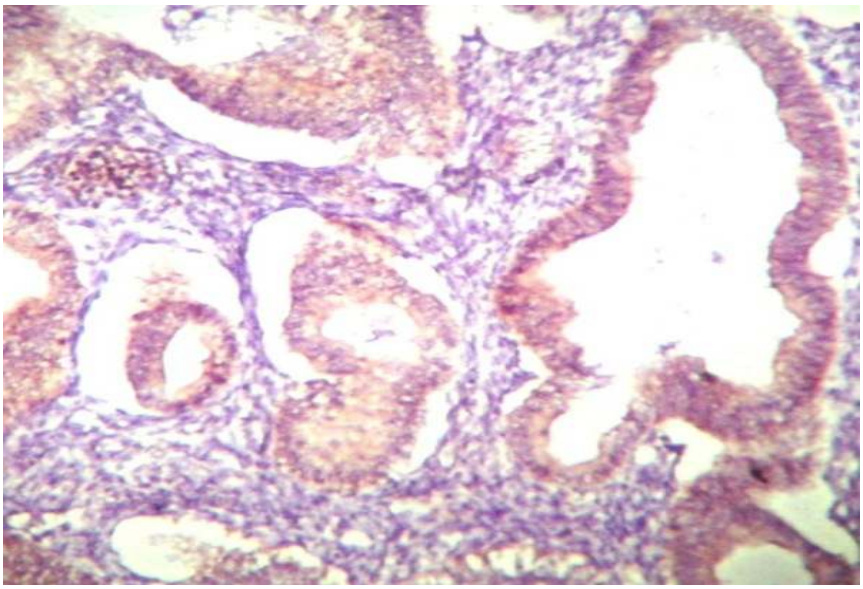


Fig-2, Proliferative phase showing cytoplasmic positivity of Bcl-2 grade-2, 10X.

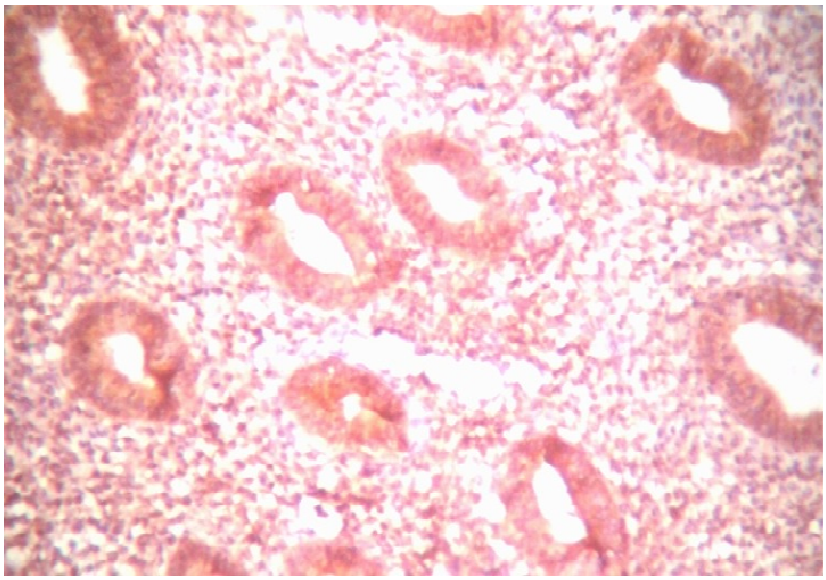


Fig-3, Proliferative phase showing grade 3 cytoplasmic positivity of Bcl-2,10X

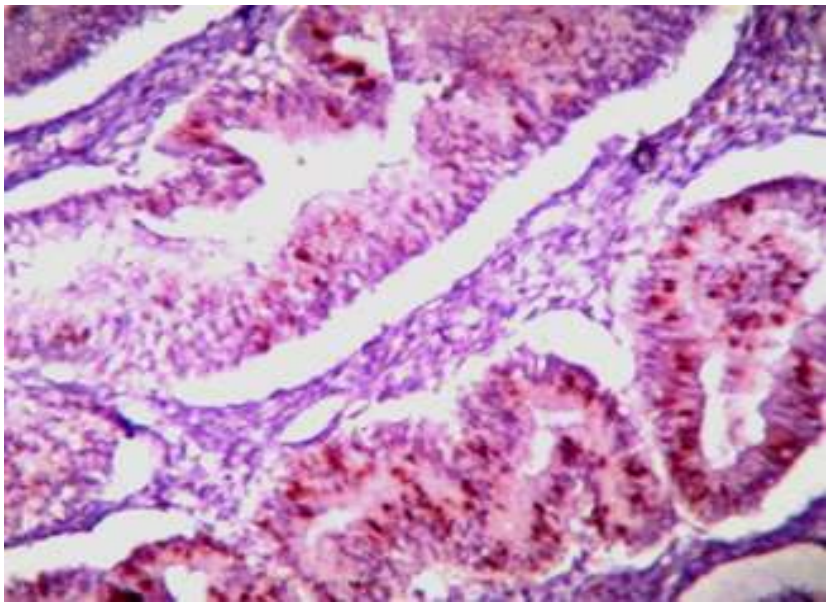


Fig-4,10X, Proliferative phase showing nuclear positivity of Ki67

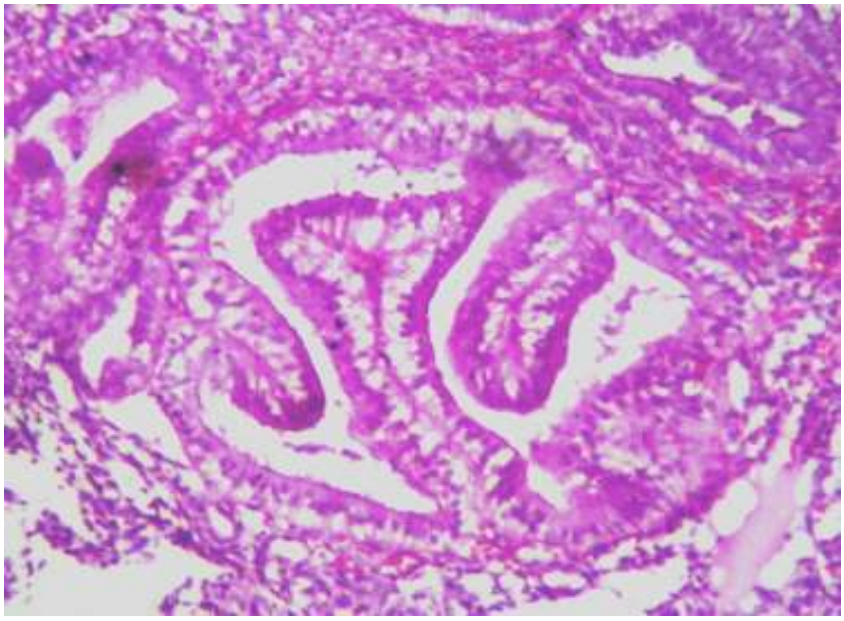


Fig-5, H&E, Early secretory phase showing subnuclear vacuoles

10X

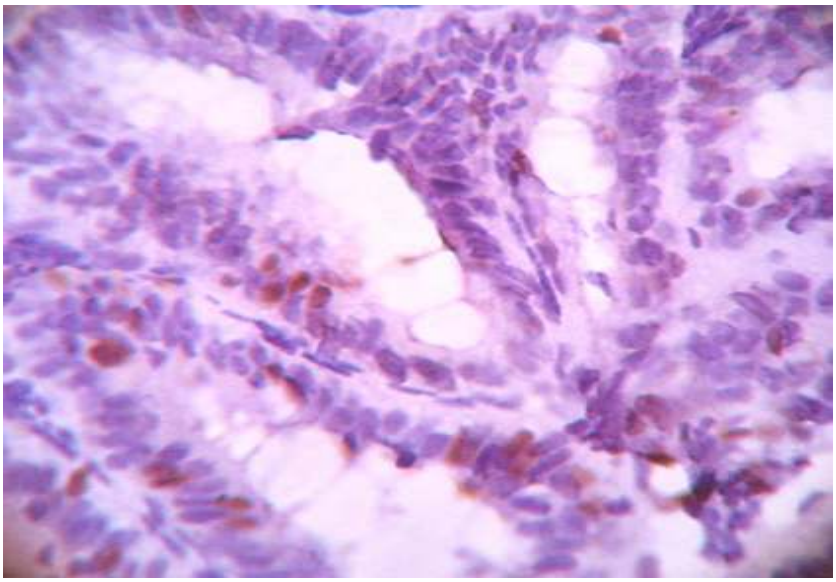


Fig-6, 10X view, Secretory phase showing nuclear positivity of Ki67

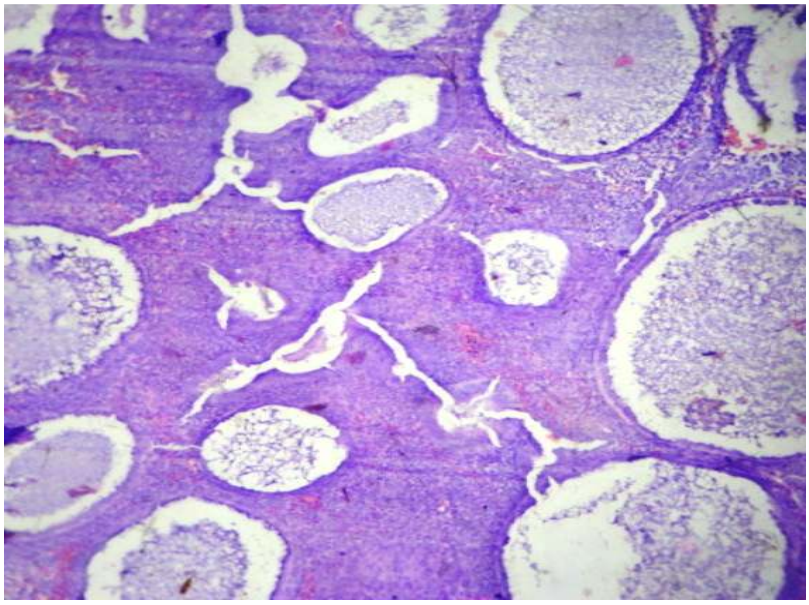


Fig-7,10X view, H&E, Simple hyperplasia showing cystically dilated glands.

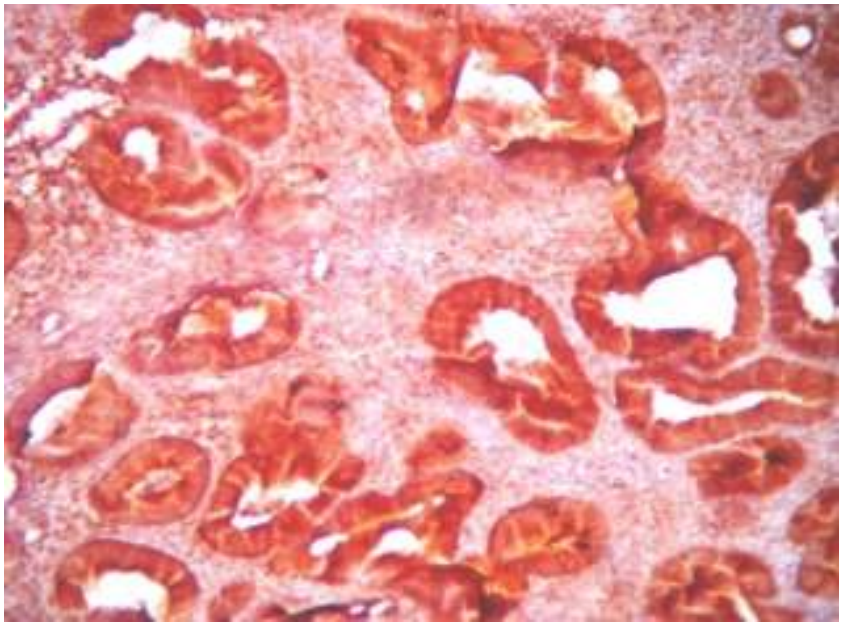


Fig-8,10X, Simple hyperplasia showing grade 4 ,cytoplasmic positivity of Bcl-2

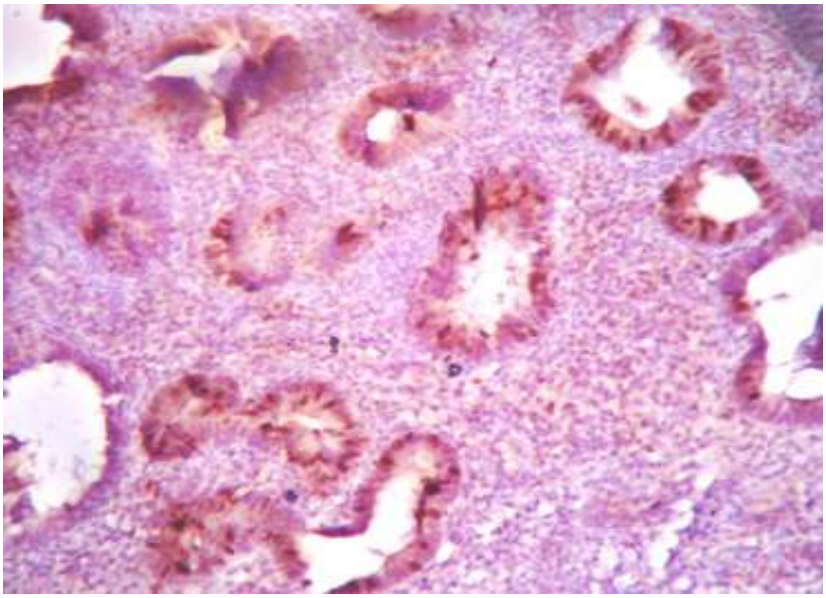


Fig-9, 10X , Simple hyperplasia showing nuclear positivity of Ki67.

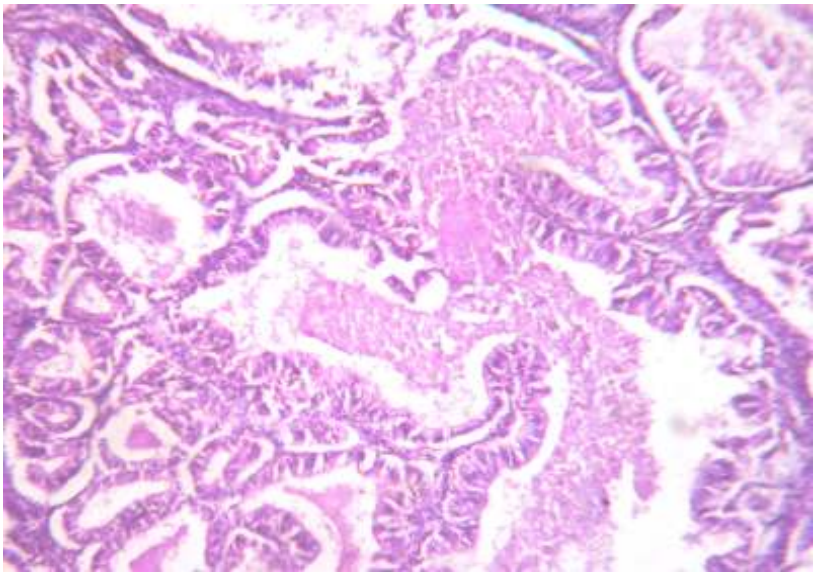


Fig-10, 10X, H&E complex hyperplasia showing complex branching glands

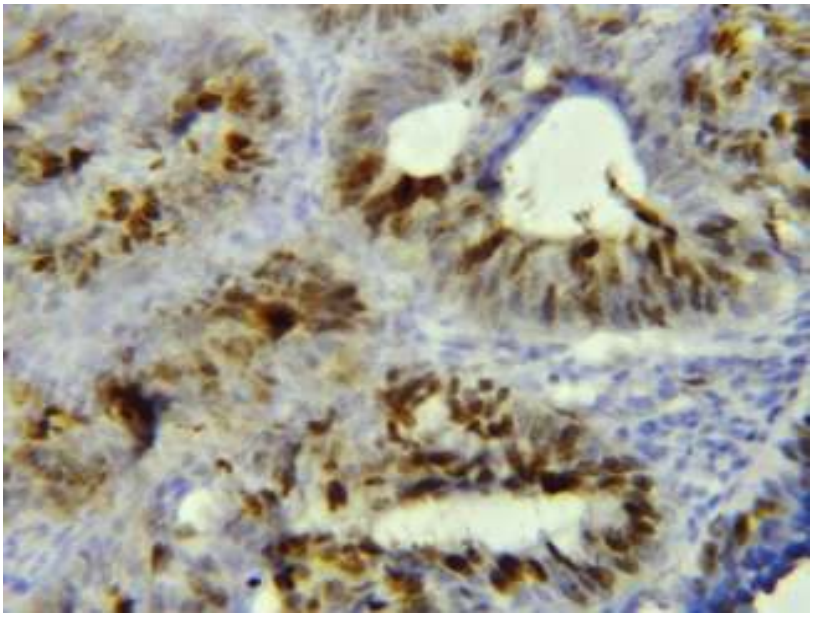


Fig-11, Complex hyperplasia with atypia showing nuclear positivityof Ki67, 10X

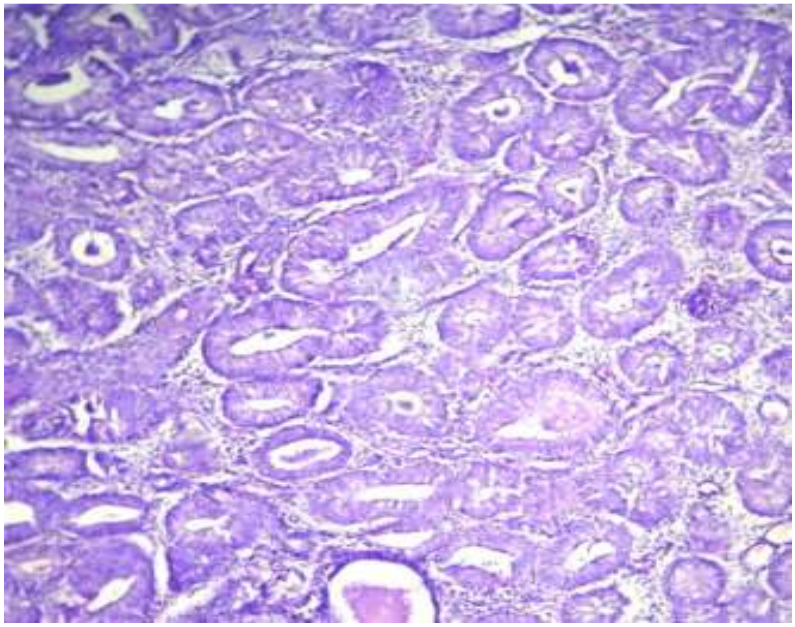


Fig-12, EIN showing focal glandular crowding with little intervening stroma, 10X.

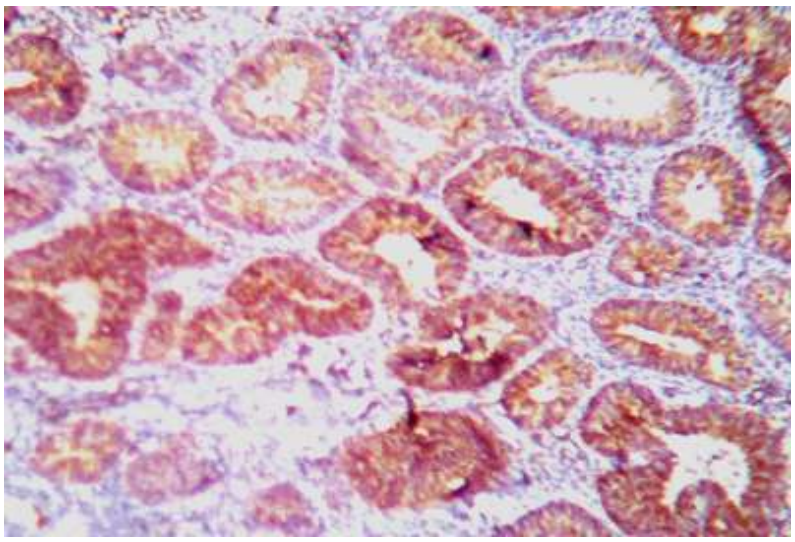


Fig-13, EIN showing grade 3, cytoplasmic positivity of Bcl-2, 10X

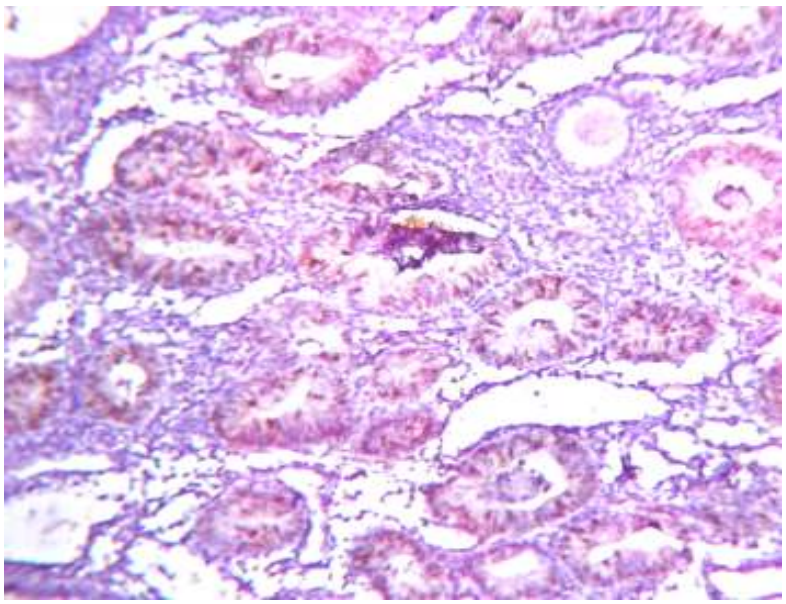


Fig-14, EIN showing nuclear positivity of Ki67, 10X.

Fig-15, Gross picture of endometrial carcinoma of uterus showing a proliferative growth filling the entire endometrial cavity and extending up to the serosa.

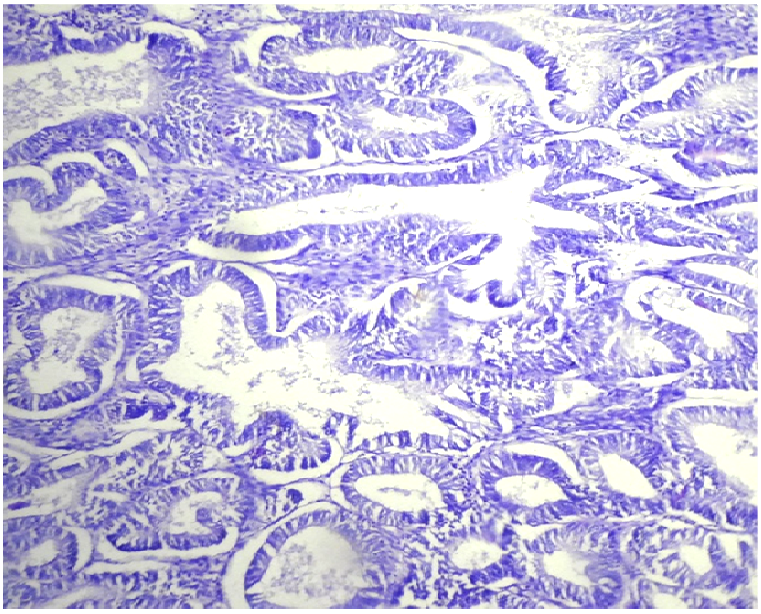


Fig-16, Well differentiated endometrioid adenocarcinoma showing back to back arrangement of glands with nuclear atypia, 10X.

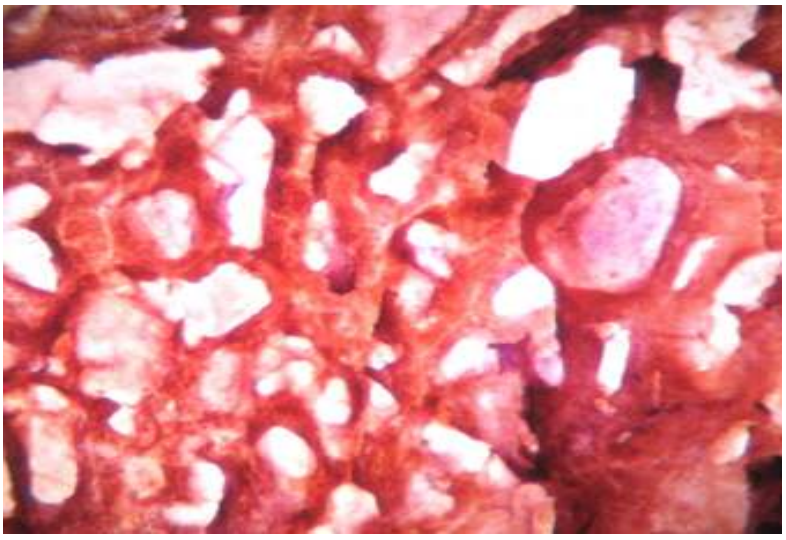


Fig-17, Well differentiated endometrioid adenocarcinoma showing grade 4 cytoplasmic positivity of Bcl-2, 10X

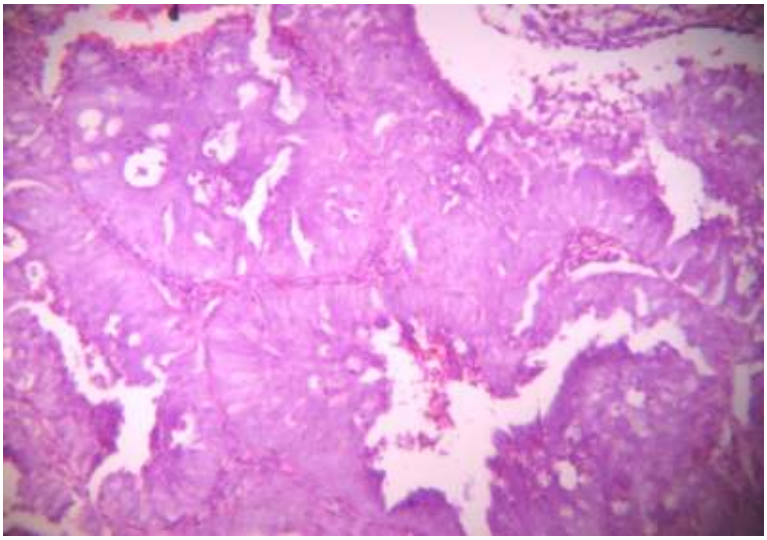


Fig-18, Moderately differentiated adenocarcinoma showing high degree of nuclear atypia. 10X

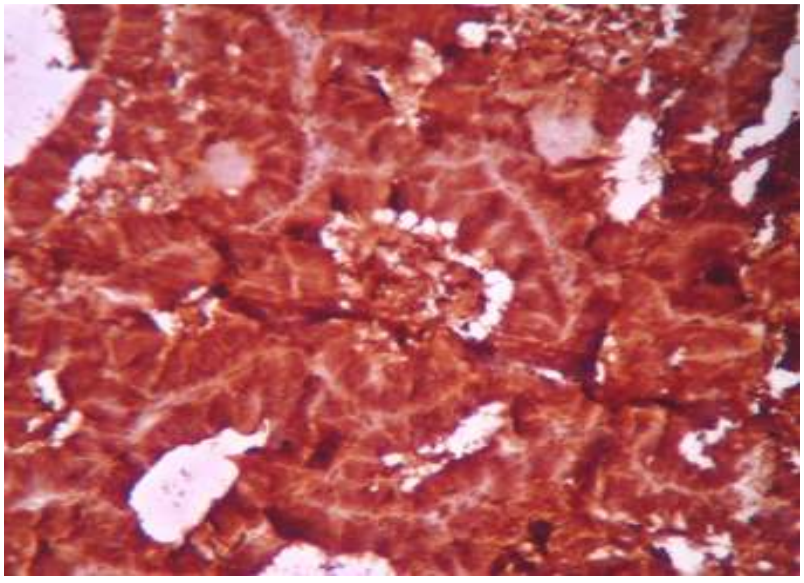


Fig-19, Moderately differentiated adenocarcinoma showing grade 4 cytoplasmic positivity of Bcl-2, 10X

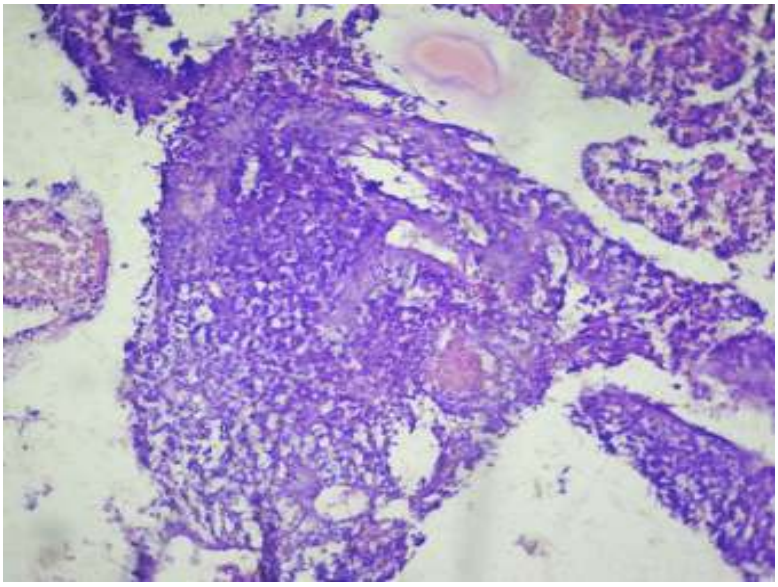


Fig-20, Poorly differentiated carcinoma showing sheet of undifferentiated cells, 10X view

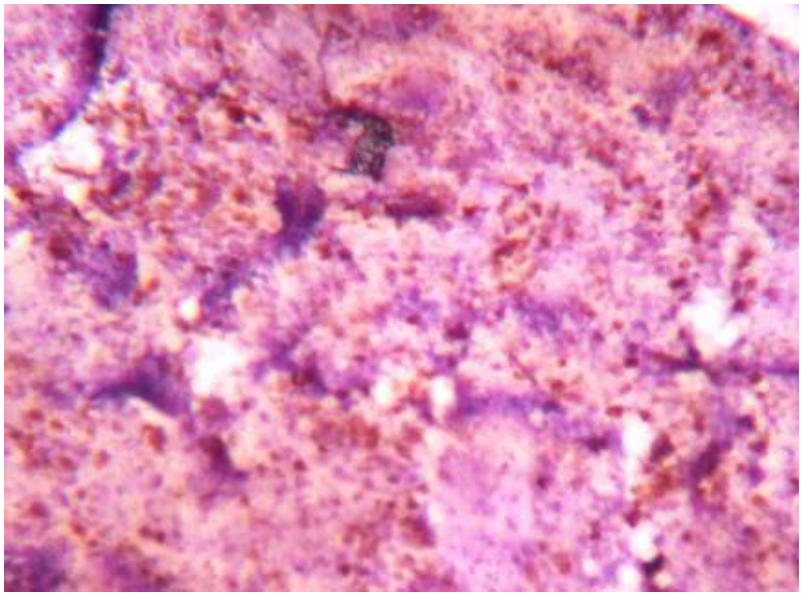


Fig-21, Poorly differentiated carcinoma showing nuclear positivity of Ki67, 10X view

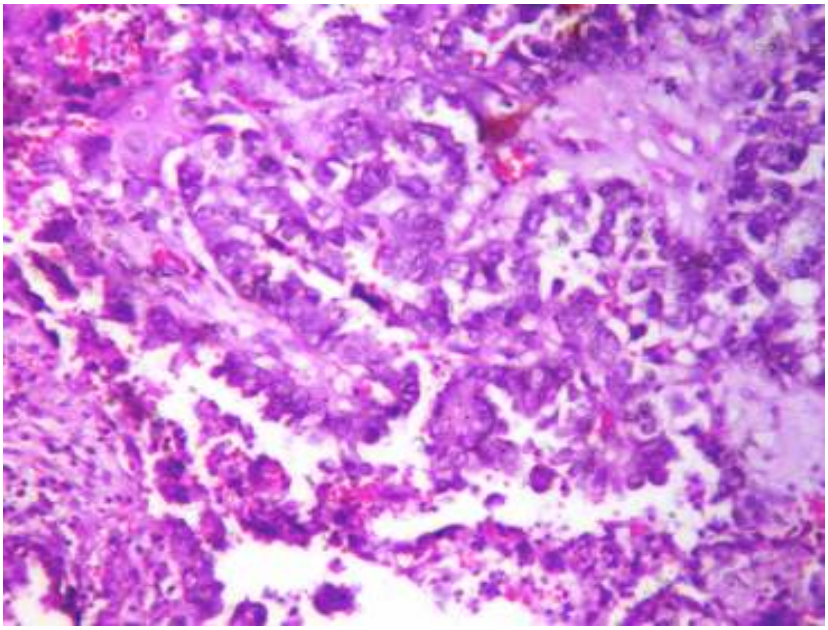


Fig-22, Serous adenocarcinoma of the endometrium showing papillary structures with cells showing high degree nuclear pleomorphism, 10X

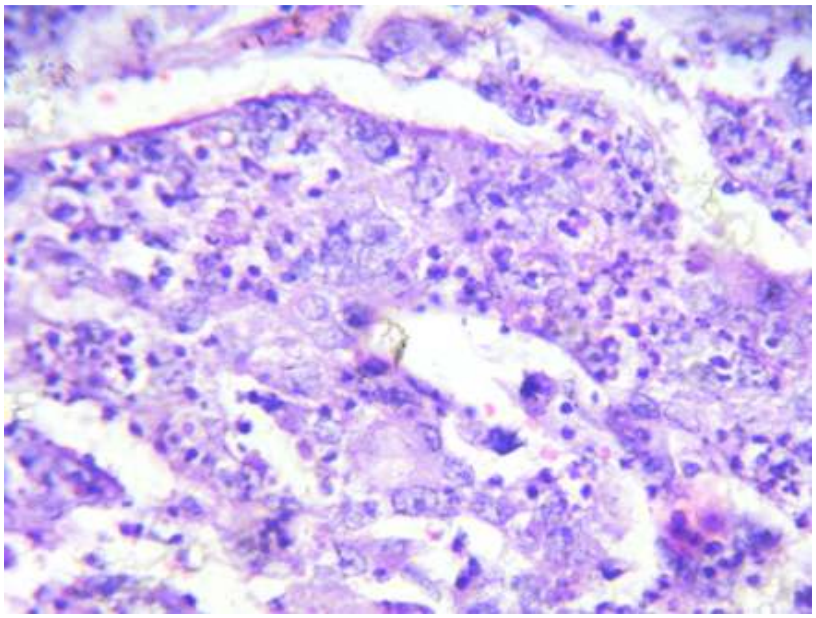


Fig-23, 40X, Serous adenocarcinoma, nucleus showing macronucleoli and apoptotic bodies

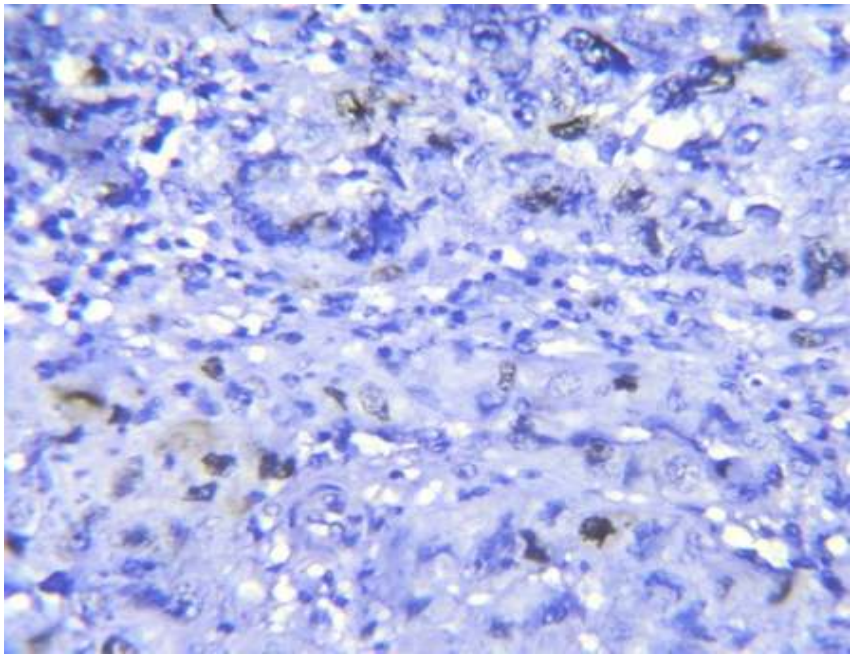


Fig 24, 40X, Serous adenocarcinoma showing nuclear positivity of Ki67

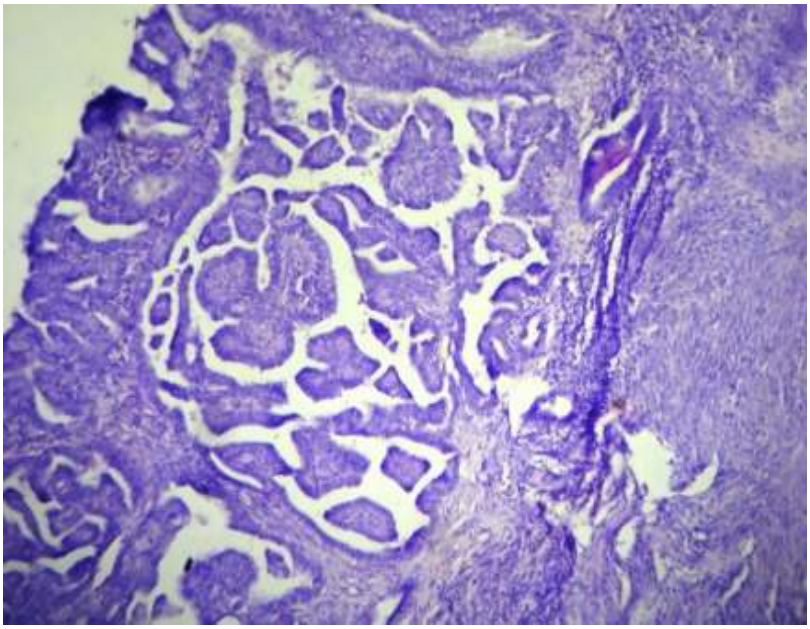


Fig-25, 10X , Serous intraepithelial carcinoma showing papillary structures resistricted to the endometrium and does not cross the endometrial myometrial junction.

DISCUSSION

A total of 50 endometrial samples were studied for Bcl-2 and Ki67 expression which included 24% of proliferative endometrium, 18% of secretory endometrium, 12% of disordered proliferative phase, 20% of hyperplasias, 6% of EIN and 20% of carcinoma endometrium.

Bcl-2 expression based on positivity alone in descending order of frequency, 100% of positivity was observed for proliferative endometrium and endometrial intraepithelial neoplasia, 83.3% of positivity for disordered proliferative endometrium, 80% of positivity was observed in hyperplasia whereas endometrial carcinoma was only 50% positive and secretory endometrium showed positivity of only 33.3%.

Ki67 expression based on positivity in descending order of frequency showed 66.6% positivity in both proliferative and endometrial intraepithelial neoplasia . 50% of positivity was expressed by disordered proliferative endometrium, hyperplasia and carcinoma, 22.2% was observed in secretory phase.

My discussion is based on the score which includes both positivity and intensity as mentioned earlier.

In cyclical endometrium, 12 cases of proliferative endometrium and 9 cases of secretory endometrium out of which 3 cases each in early,

mid and late secretory phases were studied. Mean Bcl-2 score was 9 in proliferative phase 1.77 in secretory phase whereas mean Ki67 score was 3 in proliferative phase and 0.88 in secretory phase. Thus both Bcl-2 and Ki67 expression was high in the proliferative phase and showed decreased expression in the early secretory phase. In the mid and late secretory phase both markers showed immunonegativity.

According to study done by Mertens H J MM[9] et al in 30 endometrial samples of ovulatory cyclical endometrium, Bcl2 expression was high in the proliferative phase and decreased significantly in the secretory phase, especially in the glandular epithelial cells. Ki67 also showed the same cyclical pattern with a later onset.

According to a study done by T. E. Vaskivuo[10] et al using 39 endometrial samples the results were Bcl-2 expression increased in the proliferative phase and decreased in the secretory phase being very low or absent in the secretory and menstrual phases. Ki-67 was detected predominantly in the proliferative phase.

A study done by X J Tao[11] et al states that Bcl-2 immunoreactivity was maximal during the proliferative phase and decreased in the secretory phase.

A study done by A. Gompel[12] et al in 49 endometrial samples of which 26 were proliferative endometrium and 23 were secretory endometrium and the results is that Bcl2 staining peaked at the proliferative phase and disappeared with the onset of secretory phase.

So my study results were consistent with that of these previous studies.

Comparison was done between Bcl-2 and Ki67 using paired sample t- test. The results were $p<0.01$ for proliferative phase, which was significant at 1% level. This means that anti-apoptotic activity and the mitotic activity are high in the proliferative phase.

The secretory phase showed $p>0.05$, which means not significant. The secretory endometrium which prepares itself for endometrial breakdown is characterized by increased apoptotic and decreased mitotic activity.

Out of the 6 cases of disordered proliferative endometrium Bcl-2 showed positivity in 83.3% of the cases and Ki-67 showed positivity in 50% of the cases.

Comparison of Bcl-2 and Ki67 in disordered proliferative phase using paired sample t- test gave the results $p<0.05$ which was significant. There was increased proliferative activity similar to the normal

proliferative endometrium.

Of the total 10 cases of hyperplasias, 5 cases were simple hyperplasia, 3 were complex hyperplasia and 2 cases were complex hyperplasia with atypia. Of this Bcl-2 positivity was observed in 80% of the cases whereas Ki67 showed a positivity of only 50%. Both Bcl-2 and Ki67 showed highest score in complex hyperplasia with atypia and almost equal expression in simple and complex hyperplasia.

Theodore H. Niemann[25] et al states that Bcl-2 protein expression was 100% in complex hyperplasia, 25% in complex atypical hyperplasia and 34% in cases of carcinoma. Complex atypical hyperplasia and carcinoma showed focal staining which was less in intensity than the normal proliferative endometrium.

Robert[24] et al states that, the Ki67 mean index of 68 endometrial samples in descending order of frequency were as follows. Highest expression was observed in proliferative phase followed by complex hyperplasia, atypical complex hyperplasia, and simple hyperplasia. The least score was observed in atrophic endometrium.

Morsi, Hassan[23] et el states that，107 endometrial samples studied which included eighteen cases of proliferative endometrium, nineteen cases of secretory endometrium, fifteen cases of postmenopausal

endometrium , six cases of disordered proliferative endometrium, twelve cases of simple hyperplasia, eight cases of complex hyperplasia, and twenty nine cases of endometrial adenocarcinoma. And the results were high expression of Bcl-2 and Ki-67 was observed in proliferative endometrium, post menopausal endometrium and complex hyperplasia. Secretory endometrium showed decreased expression of both the markers. In endometrial carcinoma Ki67 showed increased expression as the grade progressed, where as Bcl-2 reacted only weakly and only in grade 1 cancer.

Olga B Ioffe[27] et al states that cytoplasmic Bcl-2 expression increased from benign proliferative endometrium to simple hyperplasia and decreased in complex hyperplasia and carcinomas. Ki67 index was increased in proliferative endometrium and carcinoma and was decreased in simple and complex hyperplasia.

In my study both Bcl-2 and Ki-67 expressed highest score in complex hyperplasia with atypia, followed by simple hyperplasia and complex hyperplasia without atypia.

Correlating values of Bcl-2 and Ki-67 for the 10 cases of hyperplasia using t-test showed $p>0.05$, not significant.

3 cases of EIN was studied and both Bcl-2 and Ki-67 showed maximal expression compared to all endometrial lesions. Although the sample size is low, all the 3 cases of EIN showed 100% positivity for both Bcl-2 and Ki-67. This indicates that both anti- apoptotic activity and proliferative activity are high in the EIN, which is monoclonal proliferation of neoplastic cells. This denotes increased propensity for the EIN lesions to evolve in to cancer.

Mahrosa M.M.Khedr[30] et al states that normal proliferative endometrium expressed high levels of both Ki-67 and Bcl-2. Ki-67 positivity sequentially increased from endometrial hyperplasia through EIN to endometrial carcinoma. In contrast Bcl-2 positivity was decreased significantly in cases of endometrial carcinoma mainly the high grade.

Correlating values of Bcl-2 and Ki-67 for the 3 cases of EIN using t-test showed $p>0.05$, not significant.

In case of carcinoma total of 10 cases were studied, out of that 6 cases were well differentiated carcinoma, 1 case was grade-2 and 1 case was grade-3, each 1 case of serous endometrial intraepithelial carcinoma and 1 case of serous adenocarcinoma.

Bcl-2 expression was maximum in grade-1 and grade-2 tumours and was immunonegative for grade-3 carcinoma, serous EIC and serous

adenocarcinoma. Ki-67 mean index was 15% for grade -1 carcinoma and it was 78% for grade-2 carcinoma, 60% for grade-3 carcinoma, 42% in serous adenocarcinoma and was immunonegative for serous endometrial intraepithelial carcinoma. Thus Ki67 index increases with the grade of the tumour and also correlates with expression of serous adenocarcinoma which is around 40% according to literature[4].

Bozdogen[69] et al states that Bcl-2 expression was maximum in hyperplasia compared to carcinoma. In normal endometrium Bcl-2 staining showed increased intensity in the proliferative phase, but decreased in the early and mid secretory phase and reappeared in the late secretory phase.

Dahmoun M[70]. et al states that Bcl-2 showed no correlation to apoptotic index. The Ki67 index was higher and more heterogeneous in grade-2 and grade-3 tumours than grade-1 tumour.

Halperin R[71] et al states that the endometrioid adenocarcinoma grade-1 to grade-2 cases were characterized by increased immunoreactivity for Bcl-2, where as serous papillary endometrial adenocarcinoma and poorly differentiated adenocarcinoma also showed immunonegativity for Bcl-2.

Yamauchi[66] et al states that expression of Bcl-2 progressively decreased from low grade to high grade carcinoma and Ki67 index increased from low grade to high grade carcinoma.

Kosmas[67] et al states that Ki67 immunocytochemistry showed type-II endometrioid adenocarcinoma showed higher expression than type-I carcinoma. High grade tumours showed increased Ki67 expression than low grade tumours. Ki67 index was higher in proliferative phase endometrium than that of grade-1 and type-I endometrioid adenocarcinoma. In secretory phase the expression was markedly diminished.

Wenxin Zheng [72] et al states that Bcl-2 staining intensity was higher in the proliferative endometrium and hyperplasia, which diminished in endometrioid adenocarcinoma and serous papillary adenocarcinoma.

In my study the expression of Bcl-2 is maximum in grade-1 and grade-2 tumors and is immunonegative in poorly differentiated carcinoma and serous adenocarcinoma. This loss of Bcl-2 expression is associated with increased apoptotic activity. Serous adenocarcinoma shows increased apoptotic bodies and this correlates with the immunonegativity for Bcl-2. Thus my study correlates with the study done by, Halperin and Yamauchi et al.

Ki67 expression showed maximum expression in grade-2 and grade-3 tumours and its expression decreased in grade-1 endometrioid adenocarcinoma. This denotes that mitotic activity progressively increases with grade of the tumor. It expression is increased in high grade serous adenocarcinoma. Thus my result correlates with most of the above studies.

I observed that Bcl-2 expression decreases with higher tumour grade whereas Ki-67 index increases. This indicates increased apoptotic and mitotic activity with higher grade carcinomas.

Comparison of Bcl-2 and Ki67 in carcinoma using t-test showed $p>0.05$ i.e not significant and they show inverse relationship.

There are few drawbacks in this study which are as follows. Immunohistochemistry is a highly meticulous procedure. The antigen retrieval which is an important step is influenced by various factors such as use of old blocks, tissue fixed in formalin for long period, inadequate time of heating, pH of the buffer etc. Further if sample size is increased better results can be obtained. Since this immunohistochemistry is an expensive procedure , large sample could not be studied.

CONCLUSION

Of the 50 cases studied, in cyclical endometrium, Bcl-2 expression was maximum in the proliferative phase and decreased in the early secretory phase and was immunonegative in the mid and late secretory phases. Ki67 expression in cyclical endometrium was maximum in the proliferative phase and decreased in early secretory phase and was immuno negative in the mid and late secretory phase. This denotes that the proliferative endometrium has increased proliferative activity and decreased apoptotic activity. Comparison of Bcl-2 and Ki-67 values in proliferative phase shows a positive correlation statistically. Thus the proliferative endometrium is characterized by decreased apoptotic and increased mitotic activity in response to estrogen. This proves the association of hyper estrogenic states in causing increased proliferation leading to neoplasm.

In secretory phase as the endometrium prepares itself for shedding there is increased apoptosis which results in loss of Bcl-2 expression. Mitotic activity is also significantly reduced resulting in decreased Ki67 expression.

Disordered proliferative endometrium and hyperplasia both of which are associated with hormonal imbalance show equal and higher expression of Bcl-2 indicating the decreased apoptotic activity in these

proliferative states. Coming to mitotic index, again both of these show almost equal expression, but are lesser than Bcl-2. This denotes that these conditions are associated with an imbalance in both apoptotic and mitotic activity.

Endometrial intraepithelial neoplasia showed 100% positivity for both Bcl-2 and Ki-67 denoting the high mitotic activity and low apoptotic activity in this clonal neoplasm. This indicates the increased potential for these lesions to transform into frank malignancies.

Bcl-2 expression decreases with increasing grade of the tumour whereas Ki-67 index increases. This indicates increased apoptotic and mitotic activity with higher grade carcinoma and negative correlation of the Bcl-2 and Ki67 expression.

This negative correlation means the imbalance between the apoptotic and mitotic activity is responsible for the development of neoplastic conditions. The failure of apoptotic mechanism to remove the damaged and mutated cells combined with the proliferating ability of the neoplastic cells is responsible for the tumorigenesis.

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PROFOMA

Department of pathology

Coimbatore medical college,

Coimbatore.

Patients name:_____

Age/ Sex:_____

IP No: _____

Occupation: _____

Income: _____

Address: _____

HPE NO: _____

PRESENTING ILLNESS

History of presenting illness: _____

History of bleeding: Amount- Heavy/ moderate / scant/ variable

Duration_____

History of dysmenorrhoea: _____

History of white discharge: _____

OBSTETRIC HISTORY

Married since _____ years.

Para: _____ History of abortions: _____

Last child birth: _____

Sterilisation history: _____

MENSTRUAL HISTORY

Age of menarche: _____

Menstrual cycles: regular/ irregular, Duration:

Amount of bleeding: Heavy/ moderate / scant/ variable

PERSONAL HISTORY

Diet history: _____

Appetite: _____

Bladder and Bowel habits: _____

PAST HISTORY

History of diabetes/ hypertension/ tuberculosis/endocrine disorders/ bleeding disorders.

History of any other major illness: _____

History of drug intake: _____

GENERAL EXAMINATION

Built and nourishment: _____

Pallor /icterus/ lymphadenopathy/ pedal edema

Pulse:

Blood pressure:

SYSTEMIC EXAMINATION

Cardiovascular system:

Respiratory system:

Per abdomen: Liver- palpable/ normal

Spleen- palpable/ normal

Central nervous system:

LOCAL EXAMINATION

Speculum examination:

Vulva: Healthy / unhealthy

Vagina: presence of vaginitis/ normal

Cervix : healthy/ unhealthy

Vaginal examination:

Cervix: Direction:

Consistency:

Bleeds on touch: yes/ no

Cervical erosion: present/ absent

Uterus: Anteverted / Retroverted

Normal/ bulky/ small

Mobile/ fixed.

LABORATORY INVESTIGATIONS

1. URINE: Albumin

Sugar

Microscopy

2. HEMATOLOGY

Hemoglobin: _____gm%

RBC count: _____millions/cu mm

Total WBC count: _____/ cu mm

PCV: _____%

MCV: _____fl

MCH: _____ pg

MCHC: _____gm%

Differential count:

Platelet count: _____/ cu mm

E.S.R: _____ mm/hr

Blood group and Rh type:

Bleeding time: _____ Clotting time:

Peripheral smear :

3. BIOCHEMICAL TEST

Blood glucose: _____mg/dl

Blood urea: _____mg/ dl

Serum creatinine: _____mg/dl

4. RADIOLOGICAL INVESTIGATION

Ultrasound findings:

Others:

5. BIOPSY AND HYSTRECTOMY SPECIMENS.

Biopsy

Gross appearance:

Microscopic features:

Hysterectomy specimens

Gross appearance: Size of the specimen

Size of the growth,

Proliferative/ Irregular/ polypoidal/ infiltrative growth

Myometrial involvement, Serosal extension

Cervical involvement, Isthmic involvement,

Parametrial involvement, Vaginal cuff involvement,

Lymphnode status

MASTER CHART

S.No	HPE No	IP No	Age	Clinical Diagnosis	Histo pathological	BCL -2 Score - 16	KI 67 Total Score 16
1	G 1848/11	57731	56	DUB	PP	8	4
2	G 13351/11	24990	42	DUB	PP	8	4
3	G 1888/11	61525	40	DUB	PP	8	4
4	E 427/11	44617	45	Fibroid Uterus	PP	4	0
5	G 1357/11	41674	40	DUB	PP	16	4
6	G 1184/11	32356	44	Fibroid Uterus	PP	12	0
7	G 44A/12	97287	45	Fibroid Uterus	PP	12	8
8	G 203/12	75593	33	DUB	PP	4	0
9	G265/12	30401	45	Fibroid Uterus	PP	12	4
10	G910/12	37267	40	DUB	PP	8	4
11	G911/12	37739	29	Fibroid Uterus	PP	4	4
12	G1393/11	41447	38	Fibroid Uterus	PP	12	0
13	G1436/11	56747	54	Fibroid Uterus	ESP	8	4
14	G881/12	37147	32	DUB	ESP	4	0
15	G943/12	36667	35	DUB	ESP	4	4
16	G888/12	35878	52	Fibroid Uterus	MSP	0	0
17	G938/12	37749	38	Fibroid Uterus	MSP	0	0
18	G953/12	46080	36	DUB	MSP	0	0
19	G1767/11	48864	40	Fibroid Uterus	LSP	0	0
20	G1877/11	63431	44	DUB	LSP	0	0
21	E15/12	385	47	Fibroid Uterus	LSP	0	0

22	G2090A/11	69519	55	DUB	DPP	12	4
23	G171/12	62681	37	DUB	DPP	4	4
24	G879/12	33079	21	DUB	DPP	4	0
25	G887/12	31354	43	DUB	DPP	8	4
26	G900/12	36596	21	DUB	DPP	8	0
27	G299/12	50637	40	DUB	DPP	0	0
28	G1470/11	45129	52	Fibroid Uterus	SH	16	8
29	G1747/11	57517	40	Fibroid Uterus	SH	0	0
30	G971/11	28457	35	DUB	SH	8	0
31	G441/11	79604	41	Fibroid Uterus	SH	4	4
32	E50/12	10202	35	Fibroid Uterus	SH	8	0
33	G1926/11	65321	40	DUB	CH	16	4
34	G1193/11	38678	32	DUB	CHWA	4	0
35	E728/11	95831	43	DUB	CH	4	4
36	G175/12	6325	42	DUB	CH	0	0
37	G1608/11	51478	57	Postmonopausal Bleeding	CHWA	12	16
38	G1594/11	47678	50	Postmonopausal Bleeding	EIN	8	16
39	P472/11	80301	56	PostmonopausalBleeding	EIN	12	16
40	E728/11	9583	43	Postmonopausal Bleeding	EIN	8	0
41	G1373/11	44625	55	Postmonopausal Bleeding	EAG-1	16	8
42	G186/11	5173	65	Postmonopausal Bleeding	EAG-1	12	4
43	G426/11	4784	47	Cainomal endometaum	EAG-1	12	0

44	G65/12	4651	53	Postmonopausal Bleeding	EAG-1	0	0
45	G177/12	71940	65	Postmonopausal Bleeding	EAG-1	0	0
46	G890/12	32295	59	Postmonopausal Bleeding	EAG-1	4	0
47	G1291/11	41686	50	Postmonopausal Bleeding	EAG-2	16	16
48	G1374/11	44198	59	Postmonopausal Bleeding	EAG-3	0	12
49	G1051/11	32071	65	Postmonopausal Bleeding	SA	0	8
50	G139/12	43001	61	Postmonopausal Bleeding	SIEC	0	0

KEY TO MASTER CHART

PP	-	Proliferative Phase
ESP	-	Early Secretory Phase
MSP	-	Mid Secretary Phase
LSP	-	Late Secretary Phase
DPP	-	Disordered Proliferative Phase
SH	-	Simple Hyperplasia
CH	-	Complex Hyperplasia
CHWA	-	Complex Hyperplasia Without Atypia
EIN	-	Endometrial Intraepithelial Neoplasia
EAG 1	-	Endometrial Adenocarcinoma grade-1
EAG2	-	Endometrial Adenocarcinoma grade-2
EAG 3	-	Endometrial Adenocarcinoma grade-3
SA	-	Serous Adenocarcinoma
SIEC	-	Serous Intraepithelial Carcinoma
DUB	-	Dysfunctional Uterine Bleeding

EXPRESSION OF Bcl-2 AND Ki-67 IN ENDOMETRIAL LESIONS

ABSTRACT

The human endometrium shows dynamic morphological changes such as proliferation with high mitotic activity and secretion followed by shedding of the cells to complete the menstrual cycle. This proliferative activity is kept under check by balanced apoptotic activity.

Aim and objective : Aim of this thesis is to study the apoptotic and mitotic activity of the human endometrium by immunohistochemistry using the markers Bcl-2 an anti- apoptotic gene and Ki-67 a recognised indicator of mitotic activity. This is studied in the cyclical endometrium and various endometrial lesions such as hyperplasia, premalignant conditions and carcinoma.

Materials and methods: A total of 50 endometrial samples were studied which included dilation and curettage specimens and hysterectomy specimens received by the department of pathology, Coimbatore medical college, Coimbatore.

Results: A total 50 endometrial samples were studied which incuded 12 proliferative endometrium, 9 secretory endometrium, 6 cases of disordered proliferative endometrium , 10 cases of hyperplasia, 3 cases of EIN and 10 cases of carcinoma. The results are Bcl-2 expression was increased in the proliferative phase and decreased in the secretory phase. Disordered proliferative phase , hyperplasia and carcinoma showed almost equal expression . EIN showed maximum expression compared to all endometrial lesions. Similarly Ki-67 showed higher expression in the proliferative phase and decreased in the secretory phase. Expression of

Ki-67 in other lesions in descending order of frequency are EIN, carcinoma, hyperplasia followed by disordered proliferative endometrium. Comparison of Bcl-2 and Ki-67 in all endometrial lesions showed positive correlation in the proliferative phase and disordered proliferative endometrium, which means that there is decrease in apoptosis and increased mitotic activity in these states. In carcinoma there was negative correlation, where the expression of Bcl-2 decreased with the increase in grade of the tumour and Ki-67 expression increases with the grade of the tumour.

Conclusion: The imbalance between the apoptotic and mitotic activity is responsible for the development of neoplastic conditions. The failure of apoptotic mechanism to remove the damaged and mutated cells combined with the proliferating ability of the neoplastic cells is responsible for the tumorigenesis.